A Summary of Current Program 7/1/66

and Preliminary Report of Progress

for 7/1/65 to 6/30/66

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EASTERN UTILIZATION RESEARCH AND

DEVELOPMENT DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1965, and June 30, 1966. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C.
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INTRODUCTION

The mission of the Eastern Utilization Research and Development Division is to develop new and expanded markets for designated farm commodities, through research to develop new and improved products and processes based on these commodities. The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup and Eastern fruits and vegetables including potatoes.

In carrying out its mission, the Division does research in physical and biological science and in engineering throughout the spectrum of basic research, applied research and pilot plant development. Division scientists are aware of the key role that basic research plays in uncovering new information that may be later exploited in applied research and development. Hence, a substantial portion of the Division's effort is in basic research.

The research effort at the Eastern Division amounts to approximately 173 scientist man-years. In addition, the Division supervises domestic research contracts and grants equivalent to 30.5 scientist man-years per year at 33 locations in the United States. The Division's program is supplemented by a variety of research projects in foreign countries under PL-480 grants. There are now a total of 38 such grants in 12 countries.

The Division is organized in 10 laboratories of which 5 are located entirely at the Eastern Regional Research Laboratory, Wyndmoor, Pa., one is at Beltsville, Md., with part of its research program at Wyndmoor, and one is at Washington, D. C., with part of its research program at Beltsville. One of the laboratories at Wyndmoor has some of its research located at East Grand Forks, Minnesota, and a second has some research located at Lexington, Kentucky. One of the Division's laboratories is devoted to pioneering research on the biophysical mechanisms of macromolecules, and will be located at Brandeis University, Waltham, Massachusetts.

In every phase of their research, Division scientists cooperate with representatives of colleges and universities, state experiment stations, research institutes and associations, industrial organizations and with other Government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. Currently 11 such agreements are in effect.

The farm products with which the Eastern Division deals provide more than half of the nation's cash farm receipts; the major part of U. S. farmland suitable for cultivation is used to provide feed for livestock and dairy cattle; in seven states tobacco provides more cash receipts than any other field crop. It is thus evident that maintaining and enlarging the markets for these and the other farm products under study at the Eastern Division should be a major national concern.

Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Some recent examples of progress are as follows:

Mechanical Harvesting of Red Tart Cherries. A mechanical method of harvesting red tart cherries that was developed by Department scientists and engineers has been commercialized. The method consists of machine shaking of the tree to drop the fruit, which is caught on a padded collecting unit. Five men and one unit can replace 100 cherry pickers. The cost of harvesting by this method is one cent per pound as compared with three cents for hand-picking, and the application by Department scientists of their basic knowledge of the effects of bruising, delay and temperature on cherry quality has eliminated several problems previously connected with the processing of mechanically harvested cherries.

In 1965, 135 machines harvested 25 million pounds of cherries, (7% of the total crop) more than double the previous year's total. At a saving of two cents a pound mechanical harvesting effected a total saving of about $500,000 in harvesting the 1965 crop. Extended to the entire U.S. crop, mechanical harvesting could mean a total saving of some $7,000,000 dollars annually at present production levels. The severe shortages of labor for harvesting fruit are expected to accelerate the spread of the mechanical harvesting technique.

Chemical Properties of Cigarette Smoke Investigated. To learn more about the tumor-producing effect of tobacco smoke in animals, the alkylating properties of cigarette smoke have been studied. Alkylation is an activity common to many chemical substances, such as mustard gas and nerve gas, which can cause tumors in animal tissues. A test for determining alkylating activity in cigarette smoke was developed and it was found that smoke has relatively weak alkylating properties. Analysis of smoke fractions shows that alkylating activity is distributed throughout all smoke fractions but some fractions are more potent than others. It is probable that more than one substance is responsible for the alkylating properties. This test has been useful in following the activity throughout smoke fractionation and helpful in selecting fractions of high activity for more intensive study.

Shelf Life of Dry Whole Milk Extended. The development by Department engineers of a continuous vacuum foam-drying process for manufacturing dry whole milk has largely eliminated the fat rancidity problem previously encountered in the making and distribution of this product. In this novel process the product is made, handled and packaged without any contact with air. The process also features low heat exposure, which in the virtual absence of oxygen improves storage stability.

The air-free process has been carried out successfully under pilot plant conditions which simulate commercial operation. After a year under ordinary refrigeration the experimental dried whole milk still has good flavor. It is expected that these favorable test results will stimulate the interest of dry milk manufacturers in the commercialization of the continuous vacuum

foam drying process.

Losses in Cheese Manufacturing Reduced. Formerly many of the bacterial cultures used in cheese making were easily contaminated by bacteriophage, a virus that attacks the bacteria, interferes with its growth and prevents formation of the required acidity. A new method developed by Department scientists now prevents bacteriophage growth and activity and is in commercial use. The method, which consists of a phosphate-heat treatment of the milk used for preparing the starter cultures, is used widely by industry, particularly by Cheddar cheese manufacturers and by cheese culture supply houses. At least one company prepared its cultures in the phosphated milk and two large firms sell phosphated dry milk for use in preparing cheese starter cultures. It is estimated that the savings from use of this method in 1964 amounted to almost 2 million dollars and in 1965 to more than 4 million dollars. These figures are based on the prevention of a 1% loss in Cheddar production caused by starter failures and on a one-half cent per pound average increase in the value of the cheese made with phage-free starter.

New Cultures Increase Vitamin B. Scientists in Poland working under a PL-480 grant have developed several promising mold strains for use in making Roquefort and Camembert cheeses with increased vitamin B content. When traditional cheese-making cultures are exposed to ultraviolet light or X-rays they form mutations or new strains. Several of these mutants exhibit exceptional cheese-making qualities. One in particular, Penicillium roqueforti, has remarkable vitamin B-synthesizing abilities. It produces good quality Roquefort cheese which contains about 60% more vitamin B_2, 30% more vitamin B_6, 60% more pantothenic acid and 50% more biotin than cheese made from the patent strain. Furthermore, the new strain is much more active in breaking down milk fats and proteins during the ripening and flavor-forming stages. These new irradiated cultures have considerable promise for use in commercial cheese making.

Biodegradable Liquid Detergents Made from Tallow. Department scientists seeking a good outlet for surplus inedible fats have developed biodegradable liquid detergents from tallow. These products have excellent properties in heavy duty liquid detergent formulations. One of the new detergents is an excellent lime soap dispersing agent and can be used in liquid soap-detergent combinations for hard water use. The desirable and unexpected ease of solution of tallow-based detergents and their combination with soap will favor their commercial development. When liquid detergents were introduced in 1935 they represented only 7% of total detergent sales. Within a decade liquid detergents proved their utility and popularity. At today's levels the annual production of 14 hundred million pounds accounts for 33% of total detergent sales.

Improved Medical Pads and Paint Rollers Recapture Markets for Shearlings. Shearlings--sheepskins tanned with the wool on--are now made commercially by tanning with glutaraldehyde in a process developed by Department scientists. The shearlings are used in hospitals as bed pads for the cure and prevention of bed sores. The shearlings made by the new process resist the detanning

action of water and the deteriorating effect of alkaline chemicals so that the hospital pads have greatly improved launderability. Shearlings prepared by the new process are also used in paint rollers which resist the deteriorating action of water-based latex paints, so the rollers perform better, last longer and are easier to clean. The new process is effectively meeting the competitive inroads which man-made substitutes have been making in markets for conventionally tanned shearlings.

New Process Improves Water Resistance of Leather. Newer synthetic materials are replacing leather in shoe uppers at an increasing rate. These substitutes are promoted, in part, because of their resistance to weather and water. Easy wettability has always been a serious drawback of leather shoes. To overcome this deficiency, water-repellent agents such as silicones and fluoro-chemicals, have found application to a limited extent. Eastern Division research has developed a process that makes these agents more efficient. The process involves the retannage of chrome leather with glutaraldehyde, followed by a novel procedure for lubrication with alkenyl succinic acids. One tanner has already commercialized the process and introduced a water-proof leather for use in boots and another sportswear articles. Prospects for wider commercial adoption of the process appear very favorable.

Reverse Osmosis Applied to Maple Sap. The technique of reverse osmosis developed for recovery of potable water from sea water is being successfully applied to the concentration of maple sap. The technique consists of pumping the sap under pressure through a chamber containing a semipermeable membrane. The high pressure on one side of the membrane forces water through the membrane. Preliminary estimates of the costs to concentrate sap to sirup show a saving of 35-50¢ per gallon of sirup. A preliminary estimate for the capital investment for a reverse osmosis pretreatment plant would be approximately $20,000. Hence, a plant producing 9,000 gallons of sirup could save up to $4,500 a year over present costs. Thus the initial capital investment could be recovered within a few years. Concentration is done at or below room temperature and hence eliminates sap deterioration from heat damage. Final concentration and development of maple flavor must still be done by heating the partially concentrated sap.

The Eastern Division is one of four research divisions of the Agricultural Research Service conducting utilization research and development. The other Divisions are the Northern at Peoria, Illinois, the Southern at New Orleans, Louisiana, and the Western at Albany, California. It is evident from the examples cited above that Utilization Research can make highly valuable contributions to agriculture. Indeed, it has been estimated that for Utilization Research as a whole--adding together the contributions of the four Utilization Research and Development Divisions--more than 2.5 billion dollars has been added to the value of products made as a result of the product or process developments of Utilization Research.

Some examples of recent accomplishments of the State Agricultural Experiment Stations are as follows:

Improved Process for Producing Dry Whole Milk. An improved process for producing dry whole milk has been developed by a dairy scientist at the Pennsylvania State University. The process will produce dry whole milk with significantly improved taste and odor qualities compared with products from conventional processing. Tasty dry whole milk should increase the sale and use of milk, particularly in areas of the world where fresh milk is not available. The process has been evaluated in both industrial and government laboratories and has been found an effective means of improving dry whole milk. A commercial product based on the process is undergoing test marketing. The new process takes milk fat, separated from whole milk, and treats it with steam under a relatively high vacuum. The fat can then be re-emulsified into skim milk. At this point the product can be evaporated into dry whole milk or can be used in dry ice cream mixes or dry cream powders.

Faster Cheeses by Direct Acid Method. University of Wisconsin dairy scientists have found ways to make Cheddar cheese, cottage cheese, and Italian cheese, in a fraction of the traditional time required. Now they have done the same thing to the process of making blue cheese. Since hydrochloric acid is used to coagulate the milk, the manufacturing time is cut in half.

Lactic bacteria have not been completely replaced, since they still must be used as a starter. In the direct acid method, HCl acidifies the milk to pH 5.6, then rennet is added to coagulate the curd. After the curd is worked, lactic acid bacteria continue to increase the acidity so the pH reaches 4.8 one day after manufacture. The lactic bacteria are used to control fermentation during the curing process.

Tests indicate that blue cheese made by the direct acid method has the same texture, color, and quality, as natural blue cheese and a satisfactory flavor.

Amount of Muscle Contraction Influences Beef Tenderness. Wisconsin Station scientists, while investigating factors which make a piece of meat tender or tough, found that contraction (how much a muscle shortens after slaughter) affects tenderness. Under special experimental conditions, muscle portions were removed from the carcass and put under tension while cooling. These muscles were tender. When portions of the same muscle were free to contract as they cooled, they were tough. The extent of contraction induced by treatment was reflected by the sarcomere length. Further, a positive association was found to exist between organoleptic tenderness and sarcomere length. Shear force measurements on the cooked meat also indicated that the muscle which was allowed to shorten was much more tender than that which was permitted to contract. Although the amount of muscle contraction must now be considered one of the important factors affecting tenderness, Wisconsin scientists feel that tenderness can probably not be best explained by a single factor such as amount or kind of connective tissue, amount of fat or marbling, or sarcomere length. Sarcomere length, as a measure of contraction state, is probably only a gross indication of the molecular changes occurring in the

actin and myosin of muscle. Thus the molecular changes associated with contraction appear to be a fruitful area for further research.

A New Preserving Technique for Pork Sausage. The New Jersey Agricultural Experiment Station has discovered that a specially coated salt will extend the freshness of sausage beyond its previous limits because the coat protects against the pro-oxidant effect of salt. When salted pork sausage is frozen it becomes rancid in about three weeks. When treated with a salt coated with hydrogenated fat and frozen, the sausage is no different than before in appearance, has a genuine sausage taste after cooking and remains free of rancidity for much longer periods of time. For sausage makers, this discovery means that they will be able to make larger batches of sausage, particularly important during seasonal peak of pork production and ship them greater distances.

Apple Juice Concentration by the Sargeant Process. Virginia Station scientists have applied the Sargeant electronic concentration process to the production and characterization of a high-density apple juice concentrate. A 7.3:1 and higher density apple juice concentrate was produced without development of off-flavors. No technical difficulty was experienced in concentrating apple juice by this method to up to 82 percent soluble solids. The concentrate product reconstituted easily with water to yield a single-strength juice of good organoleptic quality. The process requires the use of additional equipment and therefore is at a disadvantage in this respect.

As a step toward implementation of the recommendations for a National Program of Research for Agriculture made jointly by the Association of State Universities and Land Grant Colleges and the USDA, a section has been added to each of the areas in this report. It comprises a list of the related publications of the State Agricultural Experiment Stations in addition to those heretofore reported covering the results of USDA and cooperative research. In future years, it is anticipated that information will be available to permit reporting of achievements resulting from State research in a format comparable to the present reporting of the USDA and cooperative research.

AREA NO. 1. DAIRY UTILIZATION--FOOD

Problem. Utilization research on milk is conducted in order that the milk products industry can defend and expand its markets, and to reduce price support expenditures by bringing commercial demand for milk products into better balance with supply.

The importance of this research can be gauged from the vast size of the industry,for dairying is one of the largest segments of American agriculture, milk is the base of an enormous processing and distributing systems, and milk is nutritionally most important:

Farm cash receipts from milk and cream marketings provide more than five billion dollars a year, about 13% of all cash receipts from farm products; only livestock marketings are greater.

The retail value of milk is eleven billion dollars a year.

Milk production is over sixty million tons a year; dairying is first in farm income in 9 states, second in 6, third in 7 and important in all the rest.

The farmer's share of the consumer's food dollar spent on dairy products averages 44 cents whereas the average value for all foods is about 38 cents.

Milk contributes about a quarter of the protein in the American diet and most of the calcium, phosphorous and vitamin B_2.

Per capita consumption of milk in the United States has been decreasing steadily and is now about 585 pounds per year, well below that of many foreign nations, including Finland, Ireland, New Zealand, Canada, Australia, Sweden, Denmark, Norway, Switzerland, Belgium and the United Kingdom, all of which consume more than 800 pounds per capita. It is thus evident that there is opportunity to increase milk consumption, despite the tremendous variety of ways in which our populace can spend its money, and the freedom it has in deciding what or what not to buy.

Even though milk production is only about six per cent above commercial demand this surplus has led to substantial government price support and related programs. Expenditures for these have ranged from 300 to 700 million dollars a year during the past five years.

Milk is a biological secretion and a valuable human food but basically it is a complex mixture of complex chemical substances. Finding out what these substances are, what their individual properties are, how to speed up, slow down, direct and, in sum, control their reactions is vital to practical

development of new markets based on new products or processes, improvement in existing products or processes, or cost reduction in existing processes.

The lead time to such development may be several years. This kind of research is recognized as a province of government laboratories, state and national, since it is basic to the whole milk industry and the results are freely available to all. Outside public research, such studies could be undertaken only by the very largest industrial laboratories and these would, quite understandably, disseminate results only as they saw fit and doubtless for their own profitability.

Government properly sponsors only research and development that the milk industry can not be expected to do for itself: projects of long duration requiring larger resources, having a substantially greater element of risk or, as with basic research, lacking the prospect of full private exploitation.

Government research and development on processes and products normally proceeds to such a technical stage that industry can logically decide whether or not to adopt them and, when adopted, government may properly also provide technical advice during the first stages of commercial application.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists and engineers engaged in basic research on the composition and properties of milk and in applied research directed to the development of new and improved dairy products and processing technology. The Department's research facilities are located in Wyndmoor, Pa., Washington, D. C., and Beltsville, Md. In addition, arrangements have been made for conducting some Division research work at Brandeis University, Waltham, Mass., beginning in F.Y. 1967.

The Federal (USDA) scientific effort devoted to research on milk totals 63.1 scientist man-years which includes 6.8 scientist man-years in the domestic contract and grant research program. This effort is distributed as follows:

(a) Chemical Composition and Physical Properties. Research at Wyndmoor and Washington totals 22.5 scientist man-years, devoted to the interaction of milk proteins in solution; mammalian and bacterial ribosomal nucleic acids; composition and structure of protein components of milk by the use of enzymes; the behavior of milk enzymes; properties of the various caseins present in milk; allergens in milk; and sol-gel transformations in milk concentrates. Contract research at the University of Maryland, College Park, (0.5 scientist man-year) deals with the relation of milk fat composition to the diet of the cow. Contract research at the University of Minnesota, St. Paul, (0.5 scientist man-year) is concerned with the possible role of genetics in affecting the heat stability of individual milks. Contract research at Ohio State University, Columbus, (0.6 scientist man-year) deals with the calcium phosphate complex in milk and milk concentrates. Grant research at North Carolina State University, Raleigh, (0.7 scientist man-year)

deals with physical changes in milks due to steam injection. In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:

1. Indian Institute of Science, Bangalore, India, on phosphoproteins of milk (5 years, 1963-1968).

2. National Dairy Research Institute, Karnal, Punjab, India, on the proteose-peptone fraction of milk (5 years, 1963-1968).

3. Israel Institute of Technology, Haifa, on the formation of unnatural nucleic acids (3 years, 1965-1968).

4. Institut National de la Recherche Agronomique, Paris, France, on the non-protein nitrogenous substances formed from milk proteins during various industrial treatments (5 years, 1961-1966).

5. Centra de Recherches sur les Macromolecules, Strasbourg, France, on the subunit structure of nucleic acids (5 years, 1961-1966).

6. University of Uppsala, Uppsala, Sweden, on the development of methods for purification of milk proteins and studies of their structure (5 years, 1963-1968).

7. Instituto Nacional de Tecnologia, Rio de Janerio, Brazil, on the structure and properties of proteolytic enzymes (5 years, 1961-1966).

8. University of Graz, Graz, Austria, on the structures and interactions of nucleic acids by means of small angle X-ray studies (5 years, 1966-1971).

During the year, research at the Institut National de la Recherche Agronomique, Paris, France, on the proteolytic activity of rennin on casein was completed, as was also research on selected enzymes of milk at the National Institute for research on Dairying (University of Reading), Shinfield, Reading, England.

(b) Flavor. Research at Washington involves 3.0 scientist man-years devoted to the study of stale flavor in sterile milk. In addition, grant research at the University of Maryland (0.5 scientist man-year) is devoted to milk flavors and flavor precursors that are derived from pasture or dry feeding practices. Grant research at Oregon State University, Corvallis, (1.0 scientist man-year) is directed to isolation and identification of specific flavor contributing compounds in butter. Grant research at the Pennsylvania State University, University Park, (0.9 scientist man-year) is devoted to study of the origin

and control of lactones, methyl ketones and their precursors in milk as a basis for the development of procedures to avoid the undesirable flavor effects of these substances in milk products.

In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:

1. National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in milk and milk products and their relation to cooked flavors and oxidative stability (5 years, 1963-1968).

2. Biochemical Institute, Helsinki, Finland, on dietary factors controlling flavor in milk (5 years, 1964-1969).

(c) Color, Texture and Other Quality Factors. Research at Washington totals 2.0 scientist man-years devoted to investigation of the allergens of cow's milk. Contract research now beginning at the Pet Milk Company, Greenville, Illinois, will deal with the stability of commercial fluid milk during refrigerated storage over periods great enough to permit the radioactivity of iodine-131 to decay to harmless levels.

(d) Microbiology and Toxicology. Research at Washington involves 1.1 scientist man-years devoted to study of the chemical and physical mechanism of the development and maintenance of heat resistance and dormancy in bacterial spores. Contract research at the University of Wisconsin, Madison, (0.2 scientist man-year) is concerned with the effects of nonfat dry milk on bread yeast fermentation. In addition, research under a PL-480 grant is in progress at the U.P. Agricultural University, Pantnagar, India, on factors which influence the synthesis of dipicolinic acid in bacterial spores (5 years, 1966-1971).

(e) Technology - Process and Product Development. Research in process and product development totals 27.7 scientist man-years at Washington, Wyndmoor and Beltsville. Studies on the preparation of dry whole milk by the vacuum foam-drying process (Wyndmoor) and the foam spray-drying process (Washington) totals 18.3 scientist man-years. The development of improved dairy processing equipment involves 1.4 scientist man-years at Washington. Development of new cheese products involves 4.5 scientist man-years and new product development based on butter fat, 3.5 scientist man-years. Contract research at the Producers Creamery Company, Springfield, Missouri, is concerned with development of a commercial scale process for removing radioactive strontium from fluid milk. This contract, supported equally by the Eastern Division and the U. S. Public Health Service, involves a total of 3.8 scientist man-years.

Also, the Eastern Division is contributing support to a U. S. Public Health Service contract with the Chemical Separation Corporation, Oak Ridge, Tenn., for research on the removal of radioactive contamination by use of a moving resin bed; the USDA contribution is equivalent to 1.2 scientist man-years.

Additional research sponsored by the Department under PL-480 grants is in progress at the following institutions:

1. National Dairy Research Institute, Karnal, Punjab, India, on the isolation and use of milk coagulating enzymes for cheese manufacture (5 years, 1962-1967).

2. Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, on the addition of nonfat dry milk solids to buffalo milk in the manufacture of hard cheese (5 years, 1961-1966).

3. College of Agriculture in Olsztyn, Olsztyn, Poland, on mechanisms of the cheese-ripening process (5 years, 1963-1968).

4. National Dairy Research Institute, Karnal, Punjab, India, on the role of starter bacteria and some genetic variants in the development of flavor during the manufacture of cheese (5 years, 1966-1971).

5. "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, on the thermal and related physical properties of milk and milk products (5 years, 1964-1969).

6. Technical University Berlin, Berlin-West Germany, on chemical changes at the surface of fat globules in foam-dried whole milk (4 years, 1963-1967).

Research on the development of mutant strains of molds with increased ability to synthesize vitamin B in cheese, at the Institute of Dairy Industry, Warsaw, Poland, has been completed.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 88 scientist man-years is devoted to this area of research.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

The application of optical rotatory dispersion and infrared techniques to the three known genetic variants of β-lactoglobulin suggests that the native protein has a structure consisting of 10-15% right-handed alpha helix, 35-40% beta conformation and the rest in a disordered state. This represents the first reported instance of beta structure in a native globular protein. The application of the circular dichroism technique to β-lactoglobulin provides data agreeing with the optical rotatory dispersion observations. Hence, β-lactoglobulin is the first protein of complex structure whose conformation in solution has become understood.

Studies on the structure of ribosomal ribonucleic acid (H-RNA) show that H-RNA from mammalian sources contains two components with molecular weights of two million and five hundred thousand, and bacterial H-RNA's have components with molecular weights of one million and five hundred thousand.

This basic research on the nucleic acid component of ribosomes is helping to describe the factors that control the synthesis of milk proteins.

In contract research at the University of Minnesota a maximum was found in the pH stability curve of bulk milk at pH 6.55 to 6.65. This maximum was eliminated if the skim milk was dialyzed against a salt mixture simulating milk salts. Thus an unknown dialyzable compound of milk apparently was responsible for loss of the maximum. Studies thus far have not shown a relationship between the heat stability of cow's milk and the genetic type of α_s or β-casein, apart from the rare α_s-casein type AA.

Continuing study of the enzymes of milk has resulted in the chromatographic separation of the four ribonucleases (A, B, C and D) and also their separation by disc electrophoresis. Major emphasis has been placed on ribonuclease B. Ribonuclease B contains mannose, glucosamine and galactosamine. Pancreatic ribonuclease B lacks galactosamine.

Digests of α_s-casein B and α_s-casein C show one peptide with different amino acid composition. It is suspected that the pair of difference peptides have the glycine-glutamic acid substitution that has been found by amino acid analysis. The α_s-casein type A is most similar to the B type but several difference peptides were found, as expected from the amino acid compositions. Similar studies done with β-casein types A, B, and C were less clear-cut.

The components of kappa-casein differ chiefly in carbohydrate content. Kappa-casein is the principal agent for stabilizing the colloidal casein in milk and although the mechanism of stabilization is not yet completely known it is clear that mechanisms which have been proposed are incorrect. Kappa-casein appears to interact strongly with polyanions, suggesting that the structure of kappa-casein is considerably different than the structure of other proteins. Small casein micelles contain a higher percentage of kappa-casein than large ones do. This suggests that kappa-casein may be in the surface of the micelle.

Two additional variants of β-casein were discovered by typing at pH 3.0. The detection of polymorphisms in milk proteins depends on the sensitivity of the method of typing that is used. As new techniques for this purpose are developed it is likely that new variants will be discovered. Already, improved techniques show the existence of a genetic variant of the red protein (lactotransferrin) in the milk of individual cows.

Research on the fat-plasma emulsion of cow's milk shows that during reconstitution of dry milk the fat-solid system in milk powder fractionates at the air-water interface and forms a fat-protein film which prevents the wetting of further added milk powder.

Grant research at the Ohio State University Research Foundation, Columbus, indicates that nuclear magnetic resonance spectroscopy is a useful tool for studying bonding within milk micelles. The binding of kappa-casein by simple anions produced marked changes in the NMR spectrum of kappa-casein. When

α_s-casein interacted with simple anions similar phenomena were not observed.

When whole milk and a 3-to-1 concentrate were sterilized at 141°C. for seven seconds, or at 149°C. for one second, the phosphatase, lipase and xanthine oxidase were inactivated. Only phosphatase showed reactivation upon storage. The degree of reactivation of phosphatase depended on the time, storage temperature and preheating and sterilizing conditions. When the whole milk and concentrate were homogenized at 4000 p.s.i. after sterilizing no enzymes showed significant reactivation.

Grant research at North Carolina State University, Raleigh, continues to demonstrate that there are severe strains at the steam-milk contact point in steam injectors. No thermal sensor has yet been found able to withstand the strain imposed by the entering steam. These difficulties demonstrate the highly dynamic conditions that exist in milk during steam injection heating.

Contract research at the Maryland Experiment Station suggests that cows receiving limited amounts of forage produced less fat than others, as shown by both total fat production and fat percentage in the milk. The average protein and solids-not-fat production and percentage composition showed no striking differences between the two groups of cows.

Investigations of the proteose-peptone fraction of milk under a PL-480 grant to the National Dairy Research Institute, Karnal, Punjab, India, show that heat treatment and sterilization (e.g. 115°C. for 10 minutes) caused a significant decrease in the proteose-peptone content of milk. A simple turbidity method has been developed for determining the amount of proteose-peptone, proteose alone and peptone alone in milk.

Research under a PL-480 grant to the Institut National de la Recherche Agronomique, Paris, France, on rennin activity shows that the action of rennin in clotting casein is notably specific. The clotting by rennin follows the splitting of a preferential bond, probably a peptide bond, and rennin probably needs a sequence of several amino acids on the casein peptide chain to recognize this bond. Rennin is not a typical protease for it is not inhibited by diisopropyl-fluoro-phosphate.

Research in progress in France under a PL-480 grant to the Centre de Recherches sur les Macromolecules, Strasbourg, on the subunit structure in nucleic acids, has led to the development of a general and rather simple method for the preparation of acid deoxyribonuclease and some other acid hydrolases. Acid deoxyribonuclease is shown to be a dimeric structure which undergoes allosteric interaction with its synthetic substrate bis(p-nitrophenyl)-phosphate.

Fundamental studies under a PL-480 grant to the Instituto Nacional de Technologia, Rio de Janeiro, Brazil, on the relation of biological activity of proteins to their structure, show that hydroxyl radicals (provided by Fenton's reagent) almost immediately modify most of the trypsin. Fractionation of the reaction mixture gives two main components, one with little

activity. The second, more active, component is not homogeneous and can be further fractionated.

Research under a PL-480 grant to the Indian Institute of Science, Bangalore, India, on phosphoproteins of milk, led to the fractionation of the peptic peptides of cow and buffalo caseins. Ten components of these caseins were thus obtained and studies of the homogeneity and characteristics of these components are now going forward.

Investigations at Institut de la Recherche Agronomique, Paris, France, under a PL-480 grant, on the nonprotein nitrogenous substances formed from the protein during various industrial treatments of milk have shown that the heating of casein liberates at least two peptides. The nonprotein nitrogenous substances liberated by heat contain more phosphorous than those obtained by an enzymatic procedure, and part of this phosphorous is in an organic state. The formation of inorganic phosphate seems to be a second step of the degradation process.

B. Flavor.

Continuing research on the stale flavor of sterile concentrated and dry milks resulted in the discovery that the material kynurenine is a possible precursor of 0-aminoacetophenone, which is in turn believed to be in part responsible for the stale flavor of concentrated and dry milks. Development of the color reagent, 2,6-dinitrophenylhydrazone of pyruvyl chloride (DNPHPC) continued, and its esters with primary, secondary and tertiary alcohols were prepared. Preparation of these esters will aid in evaluating the hydroxy-containing precursors of the lactones which form in heated milk and which are responsible for development of some of the stale flavor.

Application of gas chromatographic techniques in grant research at Oregon State University, Corvallis, has provided aromagrams of distillates of molecular and steam distillation of butteroil. The complex nature of butter flavor is indicated by the many peaks having unrelated odor characteristics. Some of the major peaks which have been identified are dimethylsulfide, delta and gamma lactones, methyl ketones and low molecular weight aldehydes, alcohols and esters. Many other flavor compounds remain to be identified.

Grant research at the Pennsylvania State University, University Park, on the origin of lactones in milk showed that the diet of the cow influences the lactone potential of the milk. Heat-treated corn diets gave 25% less lactone potential than normal diets. A quantitative procedure for determining lactones using gas liquid chromatography was developed and employed and a decrease in lactone potential in winter as opposed to summer milk was noted. This work indicates that the level of lactone potential in milk may be controlled by feeding practices.

PL-480-supported research at the Biochemical Institute, Helsinki, Finland, shows that cows maintained on a purified protein-free diet produce "zero" milk which serves as a basis for studying the origin of milk flavors. Milk production on the protein-free feed has increased with time. This "zero" milk contains the same nutrients as a milk from cows on normal feed. The

rumen contents of the test cows, on the basis of gas chromatographic determinations, are relatively poor in volatile substances with the possible exception of volatile fatty acids. The presence of characteristic flavor substances in milk--for example, the C6 to C12 lactones--could not be demonstrated in the rumen and it thus appears that the most important flavor components of milk are formed later on. Every day each cow gets 20 pounds of compressed briquets containing purified starch, cellulose, sucrose, and urea and ammonium salts, 8 pounds of a wet paste rich in cellulose and small amounts of corn oil and commercial preparations of vitamins A, D and E. Cellulose strips impregnated with silicic acid are used to improve rumination.

In research conducted under a PL-480 grant at the National Dairy Research Institute, Karnal, India, a simple and specific method for using N-ethyl maleimide was developed for estimating the free sulfhydryl groups in milk. Studies on the various ions indicate that cupric and ferric ions accelerate the rate of oxidation of the sulfhydryl groups to disulfide compounds. Hence, these metallic ions should not come in contact with milk and its products. Some of the components responsible for cooked flavor in milk were found to be dialyzable. Studies on the hydrogen sulfide content of milks show that thermal processing at high temperatures for long periods is likely to give products with hydrogen sulfide flavor and resulting poor consumer acceptance.

C. Color, Texture and Other Quality Factors.

The antigenic specificities of immunoglobulins from bovine serum, colostrum, milk, and pseudoglobulin from milk were compared by gel diffusion and immuno-electrophoretic techniques. The immunoglobulin of bovine serum and colostrum were shown to be identical. The immunoglobulin and pseudoglobulin from milk exhibited identical specificity but differed in electrophoretic mobility. A new method was devised for the electrophoretic fractionation of the castor bean allergen, CB-1A. With the new procedure the major and principal minor antigenic specificities of CB-1A were separated and estimated as 85% and 15% respectively. The previous conclusion that chemically distinct components of CB-1A contain similar or identical antigenic specificities was thus reaffirmed.

Investigations on castor bean allergens have been terminated.

D. Microbiology and Toxicology.

In continuing research on the occurrence of dipicolinic acid (DPA) in bacterial spores, spores treated with hydrazine were imbedded, thin sectioned, stained and observed in the electron miscroscope. No detectable structural differences were observed in electron micrographs of the hydrazine-treated spores as compared with the control. Chemical analysis showed no loss of calcium, phosphate, DPA glucosamine or ribonucleic acid. Germination of spores may be triggered by minute amounts of some unidentified substance. DPA is not released from the spores during electrodialysis, suggesting that it is held by covalent bonds or may exist in salt form in portions of the spore that are inaccessible to water.

Contract research at the University of Wisconsin on the use of nonfat dry milk (NDM) in continuous breadmaking indicates no correlation between the heat treatment of milk prior to drying and the amount of CO_2 produced in

ferments containing NDM. The pH influences carbon dioxide retention in dough and in baking; the most suitable pH range appears to be 4.0 to 6.0.

E. Technology - Process and Product Development.

1. Dried milk. Intensive study has continued on the cause of off-flavor found in foam spray-dried whole milk prepared in Washington during the summer months. In general, experiments tended to corroborate the idea that the greater air pollution in summer was responsible for the poor initial flavor of the product. Equipment for the analysis of volatile fractions from the foam spray-dried milk will be used to compare the volatiles from fresh, stale and "summer-flavored" product.

Near the end of the summer of 1965 some samples of foam spray-dried whole milk powder were produced with good initial flavor. With one of these samples the Economic Research Service again performed a consumer acceptance test using a panel drawn from Department employees. The results confirmed those obtained earlier: quality foam spray-dried whole milk apparently was as acceptable to consumers as fresh pasteurized whole milk from a commercial source. Foam spray-dried whole milk made by injection of low levels of liquid carbon dioxide into the concentrate before drying reconstituted with less foam formation.

Equipment for cooling the spray dried whole milk powder and packaging it in the absence of air made possible the packaging of a powder with less than 0.001% oxygen in the head space gas.

The foam spray-drying process has now been adopted commercially for the manufacture of nonfat dry milk of good quality. In the process, concentrates containing 60% or more of solids may be handled, in contrast to the usual 45%. The product is less fragile than conventional "instantized" nonfat dry milk and the entire procedure is more economical and produces a better product than the conventional process. The method can be used in making other dried products such as whey, ice cream mixes, malted milk, chocolate milk, phosphated milk for growing bacteriophage-free starter cultures for use in cheese making.

Continuing research intended to optimize the year-round production of whole milk powder from the continuous vacuum foam-drying process indicates that the mathematical model of the process is greatly facilitating the research by showing the response of production rate and product quality to process variables.

A possible solution to the problem of seasonal control would be the suppression of seasonal differences in foaming attributes by concentrating the milk to a relatively high viscosity. The viscosity then becomes the dominant factor in foaming characteristics. The concentrate can then apparently be controlled to give the required "boildown" during drying by adjustment of lecithin level as well as chamber pressure, gas content, feed temperature and other process variables.

A revised cost estimate indicates that vacuum foam-dried milk can be sold out of the dairy cabinet at supermarkets for slightly less than 20¢ per equivalent quart, which is almost 6¢ less than milk sold in large cities in two-quart paper containers.

2. Cheese. Making low-fat cheese on the pilot plant scale is progressing satisfactorily. The flavor of low-fat cheese can be improved by pretreating, for example, with lipase the small amount of milk fat that is used. Progress has been made in making a processed low-fat cheese from a blend of natural low-fat cheeses. The preliminary results provide a product with good flavor and slicing properties. The Economic Research Service has begun a marketing research program on natural low-fat cheese.

Considerable commercial interest has been shown in this new type of low-fat cheese. More than 50 cheese manufacturers have requested information, and upon request, samples have been provided to four leading cheese manufacturers.

Research under a PL-480 grant to the National Dairy Research Institute, Karnal, Punjab, India, indicates that rennet produced from bacterial sources makes Cheddar cheese comparable to that made by using commercial animal rennet. The protein degradation of the cheeses over a ten-month period was the same for both rennets. It was observed that frequent transfers of the bacterial isolates on milk agar produced progressive decrease in their ability to produce enzymes in the broth cultures. These cultures, however, retained their level of enzyme activity when preserved in soil up to a period of about 15 months. The ultraviolet irradiation of a bacterial strain resulted in higher enzyme production.

Research supported by a PL-480 grant at the Kaira District Cooperative Milk Producers Union, Ltd., (Anand, India) is making satisfactory progress in developing a cheese from buffalo milk standardized with low heat non-fat dry milk. Improvements in the cheese-making procedure have resulted in a great improvement in the product. Samples of the processed cheese made under the project had good body and texture. The flavor was clean and mild. All were superior to the samples submitted a year earlier.

Technologists at the College of Agriculture, Olsztyn, Poland, working under a PL-480 grant, continue to acquire information on the chemical changes during cheese ripening. The composition of ripening Tilsit cheese was studied and a number of components isolated and identified. Studies on the fermentative activity of some strains of lactic acid bacteria from the genera Streptococcus and Lactobacillus showed differences in the rates of lactose, glucose and galactose fermentation.

Research under a PL-480 grant to the Institute of Dairy Industry, Warsaw, Poland, found that some of the 45 mutants of Penicillium candidum possess greater proteolytic activity and/or greater vitamin B synthesis than the parent strain. Likewise, many mutants of Penicillium roqueforti had greater lipolytic ability than the parent strains and one had much

greater vitamin synthesizing ability. Thus, certain mutant strains should be much more desirable for use in the manufacturer of Camembert and Roquefort cheeses.

3. Liquid sterile and concentrated milk. The addition of polyphosphates to sterile milk concentrates, to prevent gelation and sedimentation, has been commercialized in the production of both evaporated and single-strength sterile milk. It was the discovery that polyphosphates have this stabilizing action that makes possible the production of high-temperature short-time sterile milks.

Four-to-one sterile concentrates were prepared, with excellent resistance to gelation; the concentrates remained fluid for several years at 70°F.

Severe agitation during heat treatment, especially near the point of heat coagulation, accelerates coagulation and causes sedimentation during storage, an objectionable defect. When, however, milk concentrates are sterilized under static conditions, as in "in-can" processing, polyphosphates effectively retard coagulation and gelation, even in concentrates which have undergone drastic heat treatment. This finding suggests the possibility of employing ultrahigh-short sterilization schedules.

4. Milk fat. Anhydrous milk fat of very high quality was made in a continuous operation. It contains less than 0.1% water and maintains good flavor during storage at 40°F. for several months. Several lots of ghee have been made under a wide variety of experimental conditions. The flavor of the product depends upon the quality of the cream or butter used and the temperature and duration of heating. The liquid fraction of the product has much more flavor than the solid fraction. The studies on anhydrous milk fat and ghee suggest that fractionated milk fat may provide a source of flavor in other food products.

Attempts to prepare large capsules (1000-1500 microns) containing milk fat within an edible membrane were not successful.

5. Removal of radionuclides from milk. Under the research contract with Producers Creamery Company, Springfield, Missouri, it has been shown that 90-95% of present environmental levels of strontium-90 can be removed from skim and whole milk without appreciably changing the flavor of the pasteurized product. In this research, jointly supported by the Department and the U. S. Public Health Service, the fixed-bed ion exchange system showed that the process is economically feasible for use on a commercial scale. Changes in milk composition are minor. The cost per quart of milk is less than two cents, depending on the quality of the chemicals used and the rate of processing. The greatest factor in the processing costs is the cost of regenerant. It has thus been shown that if it should prove to be necessary it is practicable and feasible to remove radioactive strontium from fluid milk on a

commercial scale.

A U. S. Public Health Service contract with the Producers Creamery Company, Springfield, Missouri, has as its objective the determination of the feasibility and practicality of a combined anion and cation fixed-bed system for removing both strontium-90 and iodine-131 in a continuous process. The design of the equipment for this purpose has been approved.

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AREA NO. 2. MEAT UTILIZATION - FOOD

Problem. Livestock production is our greatest single source of farm income. For the past several years over 30 percent of cash receipts from farming were derived from livestock sales. Likewise, the major portion of our land is used to grow livestock feed and forage. Hence, any research which succeeds in stimulating an increase in the consumption of meat and livestock products will have a profound effect on agriculture as a whole. For example, it is estimated that a one percent increase in meat consumption would require an increase in feed equivalent to 80 million bushels of corn.

The processing of meat and meat products also has an important effect on rural industry and rural employment. About half of our meat supply is derived from packing plants in rural areas. Many of these are small (the state of Pennsylvania alone has over 2,000 registered slaughterers) and cannot hope to maintain their own research facilities. They employ local labor, and their products are transported and sold by local truckers and business men. Thus, increases in meat consumption and improvements in meat technology will contribute to increased rural prosperity.

Increases in livestock consumption may be achieved through development of new or improved meat products, or through improved meat processing technology which results in lower costs. In addition, increases in the value of hides, animal fats, and renderers' proteins will benefit the livestock industry by providing additional revenues which could permit reduction in meat prices (thus stimulating consumption) or which could flow back through the marketing channels in whole or in part to livestock growers and feed producers. For example, it is estimated that loss of the market for hides would cause an increase of meat prices that would result in a decrease of 2 percent in meat consumption. Such a decrease would eliminate a market for feed equivalent to 160 million bushels of corn. Conversely, an increase in hide values would operate in the opposite direction and would result in greater income to the livestock industry and in increased utilization of feed grains.

Increased livestock consumption required both basic and applied research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research depends on new knowledge which must be developed in fundamental studies. Our supply of fundamental knowledge must be maintained and expanded if applied research is to be effective and fruitful. This need for basic research has been pointed out by the Animal and Animal Products Research Advisory Committee, by the Utilization Research and Development Advisory Committee, by the National Agricultural Research Advisory Committee, and by other responsible meat industry groups.

For the reasons given above, research which succeeds in increasing met consumption can have a powerful effect on American agriculture. The potential effect may be assessed from the facts that meat has a high elasticity demand

(a 1-percent drop in retail prices will result in a 0.7-percent increase in consumption); the production of one pound of livestock requires the equivalent of 7 to 8 pounds of feed grains; and the present United States consumption of meat (174 lb/person in 1964) is still below that of Australia (234 lb.), New Zealand (222 lb.), or Uruguay (234 lb.). Economists predict that the 1965 United States consumption will be lower than in 1964.

Attaining increased meat consumption and providing new technological information for small processors will require a vigorous and balanced research program. There is need for more applied research on processing and preservation, including expanded studies on increasing the efficiency of sausage production and on new dried or semi-dried and ready-to-eat products. Of even greater importance is the need for more basic research on the physical, chemical, and microbiological properties of meat to provide a fund of knowledge for future technological improvements.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists, microbiologists, and food technologists engaged in both basic studies and in the application of known principles to the solution of problems in the processing of meat and meat products. The Department's research facilities are located at Beltsville, Maryland, and at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 23.9 scientist man-years including 4.9 scientist man-years of contract and grant research. This effort is applied as follows:

(a) Research on chemical composition and physical properties involves 2.4 scientist man-years at Wyndmoor. A research contract at Louisiana State University provides for 1.0 scientist man-year to study the modification of muscle connective tissue constituents and their relationship to tenderness.

(b) Flavor research is conducted at Wyndmoor, and involves 4.4 scientist man-years. Additional research under a PL-480 grant is in progress at Gdansk, Poland, on antioxidant components of wood smoke used in meat-curing (5 years, 1963-1968).

(c) Research on color, texture and other quality factors involves 4.4 scientist man-years on investigations related to oxidation of tissue lipids at Beltsville, Maryland, and on fundamental studies of muscle pigment chemistry at Wyndmoor, Pennsylvania. The relationship between heme pigments and oxidative rancidity in cooked and frozen meats is being studied at Florida State University under a grant involving 0.7 scientist man-years.

Another grant, for research on the noncarbonyl compounds associated with rancid meat, involves 0.5 scientist man-years effort at Rutgers University. Additional research is in progress under a PL-480 grant at British Food Manufacturing Industries Research Association, Leatherhead, Surrey, England, on specific reducing systems in pork muscle (5 years, 1964-1969).

(d) Research on microbiology and toxicology of meat and meat products involves 5.4 scientist man-years at Beltsville, Maryland. In addition, contract research at Iowa State University, involving 1.1 scientist man-years, is concerned with a study of the fungi associated with cured meat.

(e) Technology - process and product development involves 2.4 scientist man-years at Wyndmoor, Pennsylvania. A research contract at Michigan State University involving 0.9 scientist man-years is for the purpose of developing new smoked meat products. Another contract provides 0.7 scientist man-years effort for research at the University of Missouri to develop new meat products for freezing. The development of new ready-to-eat meat products suitable for production in small, rural industries will be investigated under a contract at Southern University, Baton Rouge, Louisiana, and the reactions of muscle proteins as they relate to the thermal effects of meat processing and large-scale institutional cookery will be studied under a contract with Cornell University, Ithaca, New York. In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:

1. Taiwan Provincial Chung Hsing University, Taichung, Taiwan, on preparation of new semi-dehydrated type of fried meat products (3 years, 1964-1967).

2. University of Helsinki, Helsinki, Finland, on influence of fats on flavor and aroma of dried sausage (5 years, 1963-1968).

3. Central Institute for Nutrition and Food Research, T.N.O. Utrecht, Netherlands, on the use of protozoa to detect harmful substances in meat (5 years, 1965-1970).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 51 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

Differences have been observed in the stability of the enzymatic activity of pig myosin as compared to beef myosin. This is the first clear evidence obtained on the difference between species. Pig myosin with increased activity has been prepared in good yield from pig longissimus dorsi. The ultracentrifuge patterns of myosin from pig and from rabbit were similar in appearance.

In contract research at Louisiana State University, the effect of environmental conditions after slaughter has been determined on tenderness, muscle contraction and glycolysis. The state of contraction of muscle after aging is significantly related to tenderness; tenderness appears to increase with muscle relaxation during aging. No relation was found between mast cell count and tenderness.

B. Flavor.

The water-soluble extract of ground, raw meat was dialyzed and four fractions were obtained. Most of the precursors of meat flavor were contained in one of the fractions. In this fraction, twenty-two amino acids have been definitely identified and four more tentatively identified. This fraction also contains five sugars and sugar esters, lactic acid, and inosinic acid and its degradation products.

Identification of the components of wood smoke condensates was continued. During storage of the condensate, methylvinylketone disappears and methylformate and methyl acetate are formed with concomitant decrease in methanol and acetic acid.

In research under a PL-480 grant at the Technical University, Gdansk, Poland, four fractions of curing smoke isolated by standard extraction methods have been investigated for their antioxidative activity using lard as the substrate. With the exception of formic acid, none of the compounds containing only carboxyl, carbonyl and neutral groups showed any antioxidative activity, but substances possessing both a phenolic and a carbonyl or carboxyl group showed a specific antioxidative activity to this substrate.

C. Color, Texture and Other Quality Factors.

1. Rancidity. Study of the development of rancidity in fats showed that 0.03% sodium nitrite had a greater pro-oxidant effect on back fat from hogs than 4% sodium chloride, that randomized lard was more stable than its parent lard, and that a wide variation of the keto-glyceride content of back fat occurred from different lines of fat- and meat-type hogs. Crystalline Girard T hydrazones of the various monocarbonyl classes have been prepared and their properties determined for the first time.

In grant research at Rutgers University, freezer-storage studies have started on the determination of the non-carbonyl volatile components formed in raw, freeze-dried and cooked beef muscle to evaluate the nature and significance of these components as associated with rancidity in meats.

2. Meat pigments. A study of the reaction between the heme pigment of meat, nitrite or nitric oxide and several reductants shows that wide variety of reductants are capable of reducing nitrite to nitric oxide. The results indicate that the overall reaction occurs in two steps, the reduction of nitrous acid to nitric oxide and then the reduction of nitrosylmetmyoglobin.

Research under a grant to Florida State University shows that the main enzymatic pathway for reduction of metmyoglobin and oxygen to be from lactate via lactate dehydrogenase, DPN, flavolprotein and the electron transport chain. The enzymatic reduction of metmyoglobin takes places only after oxygen has been practically exhausted from the tissues. Frozen cured meats kept well with a combination of ascorbate and polyphosphate as long as ascorbate remained present. The disappearance of ascorbate heralded the

onset of rancidity.

Results of research under a PL-480 grant at the British Food Manufacturers Research Association, Leatherhead, Surrey, England, indicate that the rate of color development in curing is dependent entirely on the changes brought about by natural mechanism remaining in the meat. It has been established that muscle enzymes systems in the presence of suitable substrates are able to effect an anaerobic transfer of the nitrosyl group from nitrosylferricytochrome c to metmyoglobin, the form in which the muscle myoglobin is present as a result of oxidation by nitrite. Nitrosylmetmyoglobin, a product of the transference, is readily reduced enzymatically to the comparatively stable desired pigment, nitrosylmyoglobin, a complex of the muscle pigment myoglobin with nitric oxide which is responsible for the characteristic color of the cured product.

D. Microbiology and Toxicology.

1. Microbial lipases. The two fractions of lipase from Pseudomonas fragi are two forms of the same enzyme. This enzyme requires a water-fat interface, and has no activity on any nontriglyceride molecule. The culture filtrate from Staphylococcus aureus is able to attack both the 1- and 2-positions of triglycerides, suggesting that it may contain two lipases, one of which selectively attaches the 2-position. A technique for screening for 2-position activity has been developed using cocoa butter as a substrate, and will facilitate screening cultures for this type of activity. Selective lipase activity will be of considerable use in studying the structure of triglycerides in fats.

2. Flavor improvement in cured meats. Progress has been made in studying the effect of curing agents and temperature on toxin production by strains Staphylococcus aureus. Enterotoxin B is detectable at 6 hours and reaches maximum production at about 36 hours. The toxin is stable and will pass through a membrane filter without loss of titer. Sodium chloride and sodium nitrate reduced the growth rate of Staphylococcus aureus, but sodium nitrite had little or no effect.

Contract research at Iowa State University shows that Penicillium and Aspergillus are the predominent genera from country-cured ham, while Penicillium and Scopulariopsis are predominant on "fermented" sausages. Inoculated hams showed much heavier mole growth after six months than uninoculated controls and better texture and appearance. Organoleptic evaluation slightly favored the inoculated hams. The latter had a higher free fatty acid and a lower carbonyl content than uninoculated hams at 4 months. At six and ten months of curing the free fatty acid content of uninoculated and inoculated hams were similar whereas the carbonyl content of the uninoculated hams remained higher. The discovery of the lower carbonyl content of inoculated meats may be important in establishing flavor factors in cured meat.

E. Technology - Process and Product Development.

1. Processing research. A study of color development during the manufacture of frankfurter and bologna shows that emulsions prepared in an atmosphere of air developed color after an initial lag. When an atmosphere of nitrogen was used there was no lag. The lag was also reduced by vacuum and by the use of additives, ascorbic acid and cysteine. This lag apparently affects the time required for meat to consume the oxygen absorbed during comminution.

The heat coagulation of the relatively heat-resistant proteins in pork has been shown to be significantly affected by rate of heating in addition to final temperature. Rapid heating coagulated more protein than slower rates at the same internal meat temperature. Protein extracts of heated cured hams were fractionated into five major components, one of which was identified as acid phosphatase. Since measurement of the acid phosphatase content has been suggested as a measure of the thermal history of meat products, these investigations may furnish a basis for such an index.

Research under a PL-480 grant at the University of Helsinki has shown that the addition of Micrococci and Lactobacilli in the preparation of dry sausage have a distinct influence upon changes occurring during the ripening period. Lactobacilli produce a decrease in pH and speed up the ripening process, but frequently impart off-flavors and color defects. Micrococci do not accelerate ripening, but appear to prevent the adverse side effects of Lactobacilli. Both cause a decrease in count of undesirable bacteria during the ripening period. In pilot plant experiments, simultaneous addition of Lactobacilli and Micrococci reduce the ripening period from 19 to 6-7 days and weight loss was only 7% on an average as compared to a typical 20% weight loss.

2. New products. In contract research at Michigan State University to develop improved smoked meat products additional information was obtained on the effect of humidity in smoking fresh meats and sausages. It was also found that the smoke permeability of artificial casings of different types, and those obtained from different sources, may differ as much as three or four-fold. Analysis for carbonyl shows promise as a means of measuring the smoke penetration.

Research on preparation and storage of precooked frozen beef products continues under a contract at the University of Missouri. Chemical studies of stability during frozen storage showed that quick thawing at 104°F. resulted in losses of nucleotides. The constituents with the greatest potential as indices of quality are inosinic acid, creatine, creatinine and total extractable nitrogen.

In preparation of a fried meat product at Chung Hsing University, Taiwan, under a PL-480 grant, meat pieces are deep-fat fried after one or more preliminary steps such as enzyme (papain) action, salting or seasoning, and coating with egg albumin. Results indicate that pieces of about 1" x 1" x 1/2" fried to a moisture content of 30-40% (minimum internal temperature of 65°C.) were most acceptable. Dipping the pieces of meat in egg albumin prior

to frying improved both the texture and flavor of the product.

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AREA NO. 3. ANIMAL FATS AND OILS - INDUSTRIAL PRODUCTS

Problem. The 4-1/2 billion-pound-per-year output of inedible fats is one of the major products of the livestock industry. It also is one of major concern, because while production of animal fats has more than doubled in the last 15 years, its principal outlet (in soap) has declined sharply, and is still declining.

The best answer to the question of what to do with huge amounts of fats is to find new uses through utilization research. Already utilization research has played a leading role in finding new uses for over 1 billion pounds of animal fats, and thus helped retain markets for fats. Use of fat in animal feed which was developed through research, has now become the number one domestic use of inedible fats. There is need, however, for new uses not merely to retain or defend markets, but to expand them, and to upgrade the value of animal fats. The organic chemical industry presents a good opportunity for expanded markets, producing as it does a multitude of products--polymers, plasticizers, insecticides, herbicides, lubricants, paper chemicals--totaling 10 billion pounds. Animal fats possess "built-in" properties which make them potentially useful as raw materials to the chemical industry, but research must be done to realize this potential.

An increase of 1 cent per pound in the value of inedible animal fats would provide an additional revenue of $40 million of the livestock industry. This additional revenue will help the industry and growers in the same way as revenue from other animal products and by-products.

The attainment of an increase in the monetary returns from livestock requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the foundation of fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental facts about animal fats; composition, methods of separation of constituents, preparation of chemical derivatives of constituents and determination of their physical and chemical properties must be maintained and expanded if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, the National Agricultural Research Advisory Committee and by other responsible groups.

USDA AND COOPERATIVE PROGRAM

The Department has a broad program of basic and applied research at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out involving chemistry and physics, aimed at developing new and improved products from fats for use in industry. The total Federal

scientific effort devoted to this program is 39.9 scientist man-years, of which 4.3 are contract and grant research.

The research devoted to studies on chemical composition, physical properties and structure of animal fat amounts to 12.3 scientist man-years, of which 9.9 is at Wyndmoor. This research includes studies of composition of animal fats, the separation of constituents, the preparation of derivatives, the determination of physical and chemical properties of pure compounds and derivatives and, where applicable, computer programming of mathematical methods to expedite evaluation and interpretation of experimental data. A research contract on the chemical and physical characteristics of organic peroxides involving 0.3 scientist man-years at the University of Pittsburgh, Pittsburgh, Pennsylvania, has been completed. Research at Villanova University, Villanova, Pennsylvania, is continuing under a contract to study special interrelationships within triglyceride molecules and a contract on the X-ray investigation of triglycerides, each involving 0.5 scientist man-years. A research grant involving 0.7 scientist man-years at Storrs, Connecticut, provides for the synthesis of pure glycerides. At Lehigh University, Bethlehem, Pennsylvania, the interfacial absorption characteristics of fatty acids is being studied under a research contract involving 0.4 scientist man-years. Research sponsored by the Department under a PL-480 grant (5-years, 1966-1971) was initiated at Technical University, Gdansk, Poland, to study the thermally stable stationary phases for gas-liquid chromatography.

Research on chemical and physical investigations to improve products involves 25.7 scientist man-years at Wyndmoor and 1.9 in contracts and grants, a total of 27.6 scientist man-years.

Studies related to polymers and plastics include the synthesis of organic compounds and the preparation and evaluation of products derived from animal fat. The contract research at the University of Arizonia, Tucson, on plastics and plasticizers has been completed. In the contract research (now completed) with U. S. Industrial Chemical Company, New York, on ethylene copolymerization with unsaturated fatty acids and gum naval stores, EU shared the effort to the extent of 0.3 scientist man-years in cooperation with SU.

Compounds derived from animal fats are used as starting material for the preparation of lubricants and lubricant additives. This research is conducted at Wyndmoor.

Research on development of improved synthetic detergents based on animal fats includes preparation, testing of detergent power, and measurement of biodegradability of α-sulfo fatty acids and their esters, tallow alcohol sulfates and other fat derived materials. The high pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation is being investigated at Swift and Company, Chicago, Illinois, under a research contract involving 1.1 scientist man-years.

Exploratory investigations, employing novel and/or improved reaction techniques are conducted at Wyndmoor to provide new chemical derivatives from animal fats. A research grant with the Hormel Institute of the University of Minnesota at Austin, Minnesota, involving 0.5 scientist man-years provides for the investigation of the ozonization of animal fats.

In addition, research sponsored by the Department under PL-480 grants is in progress at the following foreign institutions:

1. Technical University, Gdansk, Poland, on kinetics and thermodynamics of fat autoxidation (5 years, 1964-1969).

2. "L. Torres Quevedo" Scientific Instruments Institute of the "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, on cocoa butter substitutes from animal fats (5 years, 1962-1967).

3. University of Bombay, Bombay, India, on the preparation and properties of long chain sulfated monoglycerides (5 years, 1964-1969).

4. Universite d'Aix-Marseille, Marseille, France, on hydroxylated fatty derivatives (5 years, 1962-1967).

5. Institut des Corps Gras, Paris, France. on autoxidation of fat at low temperatures (3 years, 1965-1968).

6. Centre National de la Recherche Scientifique, Paris, France, on polyhalogenated fatty acids and their derivatives (2 1/2 years, 1966-1968).

7. Technical University, Gdansk, Poland, on thermally stable stationary phases for gas-liquid chromatography (5 years, 1966-1971).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.1 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure.

Selected tissues (back fat, leaf fat, liver, pancreas and serum) from two breeds of pigs, Duroc and York, were examined for differences in fatty acid and/or glyceride composition. Animals were chosen on the basis of high and low percentage of back fat. Thus far there appears little difference between breeds or between level of back fat. The composition of the serum lipids resembles that of the liver in that most of the C-16 acids are found in the 1-3 position of the glyceride.

A new procedure has been devised for the quantitative preparation of the methyl esters of long-and short-chain acids for gas-liquid chromatographic analysis. The pancreatic lipase hydrolysis technique has been modified to permit satisfactory operation for high-melting triglycerides.

In contract research at Villanova University a computer program was applied to a study of the interactions between parts of triglyceride molecules which come close to each other. Fundamental information has been obtained on the internal rotation barriers of the hydrocarbon parts of these molecules.

Under another contract for research at Villanova, 9 triglycerides have been prepared. The structure of one of them (β-11-bromoundecanoyl-α,α' dicaprin) has been determined, and represents the first instance where the structure of a mixed triglyceride has been determined.

Research on the synthesis of pure glycerides continues under a grant at the University of Connecticut (Storrs). Alpha-monoglycerides were prepared easily, as were also the 1,3-diglycerides of palmitic and stearic acids. Preparing the 1,2-diglycerides was more difficult. Two pure triglycerides, 2-oleo dipalmitin and 1-stearo diolein, were synthesized and furnished to EU where they will be investigated to establish their physical and rheological properties.

Single crystal X-ray examinations were made on dibenzoyl peroxide (an example of the diacyl peroxide class) and two peroxy acids. These studies have helped clarify some of the structural features of the two classes of peroxide compounds. This research, under a contract at the University of Pittsburgh, has been completed.

Experimental methods have been developed for obtaining spectra of fat components in the crystalline state; practically all components of fat can thus be positively identified and characterized. Polarized infrared spectroscopy was developed and its use in conjunction with X-ray powder diffraction measurements provide a good way to investigate the chemical and physical structure of fats and their derivatives.

In contract research at Lehigh University examination was continued of the series of esters of α-sulfo fatty acids representing those of the detergent type and those of the wetting agent type. The methodology involved in measuring surface tension and contact angles has been improved and is now satisfactory. Detergent types of the esters of α-sulfo fatty acids tend to show a decrease in surface tension with time, whereas most wetting agent types show no change. Cross-sectional molecular areas were determined and two types show a difference in cross-sectional area.

The normal alkyl esters of long chain acids can be distinguished by X-ray diffraction measurements. Other modern techniques are being used for fundamental studies of the properties of animal fats and derivatives. Progress has been made in applying dielectric measurements to the structural

investigation of these materials. A capillary extrusion rheometer was developed for study of the flow behavior of lard. Research was continued on the research of polyunsaturated fatty acids with specific cis-trans and positional isomerism.

The development of mathematical methods employing a computer for treating experimental data from basic research continued with the development of a computer program for the dielectric constant and dielectric loss of some fatty acid esters. Also, a program was developed for investigating the possibility of hydrogen bonding between hydroxyl groups and olefinic double bonds. Likewise, work was continued on the theoretical treatment of counter-current distribution.

B. Chemical and Physical Investigations to Improve Products.

1. Plastics investigations. A series of rigid urethane foams were prepared from oxypropylated 9,10-dihydroxystearic acid. The physical properties of these urethane foams were similar to those of commercial products. Urethane foams containing fire-retardants have also been prepared and are being evaluated.

In studies related to the preparation of polymers from animal fat derivatives, data were obtained suggesting that many monomers with C-18 side chains will be efficient as internal plasticizers. Since fat-derived allylamide and allyl ester monomers can be easily prepared directly from tallow or tallow acids, N-allystearamide was copolymerized with three commercial comonomers. Co-polymers of vinylidene chloride and five acrylamides were prepared and their solution properties were determined.

Contract research at the University of Arizona, now completed, showed that vinyl stearate continues to be a promising internal plasticizer for vinyl chloride.

2. Detergents investigations. Alkyl palmitates and stearates can be alpha-sulphonated directly with liquid SO_3 in a yield of 75%. This direct sulphonation has economic advantage because it avoids the use of solvent and the need to isolate the α-sulfo acid. Combinations of saturated or unsaturated tallow alcohol sulfates with esters of α-sulfo tallow acids are synergistic with excellent solubility, foaming and detergent properties. These fat-based detergents are also readily biodegradable.

Investigation of ether alcohol sulfates was continued. The products derived from saturated tallow alcohols and propylene oxide or 1,2-butylene oxide have good calcium ion stability and lime soap dispursing power.

An analytical method for determination of sulfate ions in detergent media was devised to follow the metabolism of anionic sulfate and sulfonate detergents in their biodegradation by microorganisims.

In PL-480 supported research at the University of Bombay, a convenient method has been developed for the preparation of pure α-monoglycerides. Five long-chain (C_{12} to C_{18}) sulfated monoglycerides have been prepared.

The high pressure hydrogenolysis of animal fat to alcohols without simultaneous chain saturation is being investigated under contract research at Swift and Company. Washing experiments on tallow alcohol sulfates of known composition show that unsaturation improves detergency in cold, hard water.

3. Lubricant investigations. The alkyl and glyceryl ester of polyunsaturated acids react almost quantitatively with alkyl phosphonates to give viscous, oily products. The phosphorous-containing materials are effective hydrodynamic lubricants as base oils and also as additives to refined paraffin crude oils of synthetic oils. The evaluation of these lubricants indicate that they are superior to some commercial materials.

Several α-branched fatty acid esters were synthesized and showed considerable potential for use as specialized lubricants. Larger quantities of pure branched chain esters of fatty acids were prepared for lubricant study and evaluation.

4. Reactions investigations. The periodic acid cleavage of epoxy esters permits the direct conversion of epoxides to aldehydes and permits the determination of the location of epoxy groups on hydrocarbon chains.

The cynanoethylation of hydroxolated fatty esters and related compounds has been achieved and provides 95% yields of β-cyanoethyl ether derivatives.

The reactions of isopropenyl stearate are affected by the reaction medium. A catalyzed reaction in the absence of solvent or diluent produces stearone. In the presence of a high boiling ester, a rearrangement reaction of the isopropenyl stearate forms the potentially useful β-diketone, heneicosane-2, 4-dione. With inert hydrocarbons the product is hexadecyl ketene (stearoketene). On standing, the ketene dimerized to form 2,4-dihexadecylcyclobutyl-1,3-dione.

In grant research at the Hormel Institute the ozonization of saturated fat materials has resulted in the formation of light hydrocarbon gases and dimers, trimers and polymers of the starting material. Pyrolysis of ozonides of unsaturated material in the presence of metals in the reduced state produces aldehydes.

Investigation of the autoxidation of emulsified fats shows that anionic emulsifiers enhance the pro-oxidative action of metal ions and basic amino acids to act anti-oxidatively. The experiments indicate that the necessary condition for the pro-oxidative interaction of metal ions (Fe,Cu)with a basic amino acid such as histidine, lysine and arginine is the formation of a partially complexed metal ion.

The kinetics and thermodynamics of fat autoxidation is being studied under PL-480 supported research at Technical University, Gdansk, Poland. The experimental and analytical techniques, particularly polarographic peroxide values applied to metal oleate autoxidation has provided useful data. The data for double bond disappearance indicates that this is a first order reaction.

In PL-480 supported research as Universite d'Aix-Marseille, Marseille, France, progress continued on the preparation of hydroxylated derivatives of fatty acid. In a catalyzed reaction, oleic acid was converted to allylic monohydroxy unsaturated acids plus some by-products.

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AREA NO. 4. HIDES AND LEATHER UTILIZATION

Problem. To maintain the utilization of animal hides and skins at a profitable level there is need to find new products and processes to provide outlets for about 13 million cattlehides that are now available in excess of domestic needs. The foreign markets that currently absorb these surplus hides are also threatened by the increased hide production and decreased per capita use of leather (the principal outlet for hides) that have dislocated U.S. markets and caused prices to drop so precipitously in the last 10 years. To meet this problem there is need for upgrading the quality of raw hides and skins, for reducing the costs of producing leather, and for developing new and nonconventional products from collagen. To achieve these objectives research is needed to develop improved curing processes and agents, more effective control measures for (ante mortem) defects such as grubs, brands and parasite damage, and improved methods of take-off. Fundamental research is needed on the composition of hides to provide basic information on the chemical, physical and physical-chemical properties and reactions of collagen and other hide components for use in studies on chemical modification and on the development of new and improved products and processes. Development of new, more rapid and economic processes for curing, handling, unhairing and tanning hides is needed to reduce the cost of producing leather. There is also need for research on the chemical modification of hide proteins to develop leather products with such improved "built-in" properties as increased resistance to wear, scuffing and deterioration from perspiration, enhanced washability, dry-cleanability and improved dyeability. There is also need for research on the physical and chemical properties of collagen to obtain information for use in dispersing and regenerating the fibrous structure without degrading its unique properties for developing nonconventional products that will provide new outlets and markets for hide proteins, with special reference to the field of edible products.

USDA AND COOPERATIVE PROGRAM

The Department is conducting a broad program of basic and applied research on hides, skins and leather at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out; this involves physicists, chemists, biochemists, microbiologists and leather technologists.

The Federal scientific effort devoted to the over-all program totals 27.2 scientist man-years, as follows:

(a) Research on chemical composition, physical properties and structure of hides and leather involves 12.5 scientist man-years at Wyndmoor. One line of investigations is concerned with the isolation of collagen and other hide components and basic research on the chemistry of collagen. This research is supplemented by a grant at Northwestern University School of Medicine, Chicago, involving 0.6 scientist man-year to study physical properties of collagen and a PL-480 grant at the University of Turku, Finland, (5 years,

1960-1965), on fractionation of gelatin and collagen. Research is continuing at the University of Turku under a PL-480 grant (3 years, 1965-1968) for basic investigations on the structure, biosynthesis and maturation of collagen.

Other investigations at Wyndmoor are concerned with the relation of hide composition and structure to leather properties. Additional research is in progress at the Central Leather Research Institute, Madras, India, under PL-480 grants on (a) the hydrothermal shrinkage of collagen and leather, (3 years, 1964-1967) and (b) comfort properties of shoe leather (5 years, 1964-1969).

(b) Chemical and physical investigations to improve products involves 6.6 scientist man-years at Wyndmoor. This research is concerned primarily with the investigation of chemical modifications of hides prior to and during tanning operations to provide improved leather. A contract at the University of Cincinnati, Cincinnati, Ohio, provides 0.2 scientist man-year effort for research on the noncollagenous proteins of cattle hides. Midwest Research Institute, Kansas City, Missouri, is conducting contract research involving 0.5 scientist man-year to study the dispersion of collagen.

In addition, research sponsored by the Department under PL-480 grants is in progress at Central Leather Research Institute, Madras, India, on (a) polyphenolic tanning compounds (5 years, 1962-1967) and (b) preparation and determination of physico-chemical properties of polypeptidyl derivatives of collagen (5 years, 1966-1971), and at the British Leather Manufacturers Research Association, Surrey, England, to investigate chemically reactive compounds for improving leather stability (5 years, 1963-1968).

(c) Technology - process and products development involves 6.0 scientist man-years at Wyndmoor. Research includes development of new tanning processes for hides and skins to provide products of superior durability and development of regenerated collagen products. Contract research at the University of Cincinnati, Cincinnati, Ohio, involves 0.8 scientist man-year on the abnormalities of leather characterized by a depleted, mushy texture.

Additional research under PL-480 grants is in progress at: (1) Central Leather Research Institute, Madras, India, on (a) relation of hide quality to tanning rate (5 years, 1962-1967), (b) radioactive tracer study of mineral tanning (5 years, 1965-1970), and (c) rapid tannage of sole leather (5 years, 1965-1970); and (2) Leather Research Institute, T.N.O., Waalvijk, Holland, on kinetics of chrome tanning (4 years, 1964-1968).

PROGRAM OF STATE EXPERIMENT STATIONS

State stations reported no research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure.

1. Protein constituents and collagen. Nuclear magnetic resonance studies were initiated to obtain information concerning the fundamental factors which determine the secondary structure of collagen. NMR spectra have been obtained for several amino acids. The studies show that the stable forms of phenylalanine are not those predicted by theory. Instead, the data suggests that a single conformation predominates in which the C-C-C-N dihedral angle is approximately 30^{o}. Preliminary results with infrared studies suggests that the predominate features of the secondary structure of proteins can be satisfactorily determined in deuterium oxide solution.

When collagen is dissolved from calfskin by means of citrate buffer the concentration of collagen in the liquid phase increases and then levels off. Replacement of the buffer with fresh buffer dissolves still more collagen and this process may be repeated several times before the exhaustion point is reached. The solubilizing studies have resulted in obtaining larger yields of dissolved collagen from skins. The construction of automated equipment for solubilizing collagen has been completed. The proper conditions are maintained automatically through electrical conductivity and pH sensing elements. A water-soluble component with unusual properties has been isolated from solubilized collagen preparations. It has been found that pickled cattlehides have an extremely slow rate of equilibration with salt and acid.

In grant research at the Northwestern University School of Medicine, Chicago, additional information was obtained on the hydrogen bonding in the solvent pairs formic acid-dimethyl formamide and formic acid-N-methyl acetamide. Mutarotation studies on poly-L-proline, poly-L-hydroxyproline and poly-O-acetyl-L-hydroxyproline showed different kinetics for these materials.

In research under a PL-480 grant at the University of Turku, Finland, the optimal conditions for starch-gel electrophoresis of heat-denatured collagen were determined. The effect of long heat denaturation of collagen was studied and various subunits which resulted from this treatment were fractionated by electrophoresis. Degradation of collagen under alkaline conditions produced an unexpected subunit. Research under this grant has been completed and will be followed by another which provides for basic investigations on the structure, biosynthesis and naturation of collagen.

2. Hides and leather. Microscopical investigation of skin and leather fiber structure has revealed the structural features of two important leather defects. One,calfskin veininess, is caused by large void spaces of undetermined origin around the larger blood vessels of the grain surface. The other defect is abnormal vertical fiber structure, which leads to greatly reduced strength in resultant side leathers. Polarization microscopy was used in four different ways to study this vertical fibre defect in hides.

This technique permits more ready evaluation of the hides, and densitometry applied to the photographic films provides a quantitative expression of the degree of abnormality.

The transition of solubilized collagen from a helix to a random coil can now be measured quickly and with a small amount of sample by differential thermal analysis. The effect of moisture on the thermal and mechanical properties of leather was begun with investigations which utilized two techniques, stress relaxation and differential thermal analysis. Measurements were made of the relaxation ratio of a sample of vegetable tanned leather and of water binding by collagen in hides and leather. The effect of moisture on the thermal and mechanical properties of leather may serve as a good indication of the effect that tanning and other modifications have made in a hide.

Research in progress under a PL-480 grant at the Central Leather Research Institute, Madras, India has demonstrated that the physical changes that collagen undergoes when subjected to heat are more complicated than had been predicted. It was found that the effects of pretreatments and of cross-linking agents can not be explained by application of theories developed from studies of elastomeric polymers. It appears that studies of hydrothermal shrinkage will produce data highly significant to the elucidation of the physical properties of collagen and the behavior of leather.

The comfort properties of shoe leathers is being investigated under a PL-480 grant at the Central Leather Research Institute, Madras, India. Experimental studies have shown that leather can absorb an appreciable amount of perspiration and can give away the same in the process of evaporation, thus acting like a reservoir. The leather can also spread perspiration over its surface uniformily. Repeated flexing increases water vapor permeability and air permeability. Leathers with resin finishes have less permeability than leather with protein finishes. The relative humidity, present during actual use conditions, in various places between the foot and the shoe for a wide variety of shoes was above 65% at all places. The highest values were found in the region where the sole of the foot comes in contact with the shoe. A rubber sole, a hair-on upper or a canvas upper increased the relative humidity present within the shoe. The dissipation of perspiration and the increased permeability of leathers with use may be important properties specific for leather.

B. Chemical and Physical Investigations to Improve Products.

1. Mannich reaction. Studies on the Mannich reaction involving interaction between amine, formaldehyde and a compound with an active hydrogen indicated that malonic acid is a promising source of the active hydrogen. This reaction, which incorporates new carboxyl groups into the hide, is easy to carry out and improves fixation of chrome and alum. Research indicates that lysine participates in the reaction and that new amino acid residues are formed in the protein. The formaldehyde and malonic acid can be added together but, for best results, the treated hide must be tanned separately with chrome. The product has a high shrink temperature and has good

resistance to deterioration on washing.

2. Other chemical modifications. Experiments using reactive leather dyes demonstrated that these dyes appear to impart the property of wash fastness. This is in addition to perspiration resistance, which can be attained through glutaraldehyde tanning. Leather made by tanning with dimethylol urea plus resorcinol had excellent resistance to perspiration and is nearly white in color. A large-scale test of various types of sheep and goat skins provided good quality leather with a striking pebbled grain effect. This leather, however, has inadequate resistance to light, due to the resorcinol.

In research under a PL-480 grant with the British Leather Manufacturers Research Association, work has continued on the mode of action of aldehydes in stabilizing collagen. The use of combinations, such as formaldehyde-acrolein, glutaraldehyde-acrolein and glyoxal-dialdehyde starch revealed differences in reactivity, stability and the extent to which different protein groups are involved. Reaction of collagen with other compounds has been more difficult to achieve. Triazine derivatives, acyl chlorides, and isocyanates are insoluble and sensitive to water, and difficulties arise with diffusion through a solid fibrous material.

The encouraging results obtained with purified tannin derived from mangrove in research in progress under a PL-480 grant at the Central Leather Research Institute, Madras, India, has led to the fractionation and evaluation of other tannins. The source of phenolic tannins includes babul and dhawa. Dhawa was found to be a better material than babul for pretanning and retanning with chrome. The observation that purified babul extract has a fungicidal action is interesting since this is contrary to the usual experience.

3. Pretannage investigations. The application of an aqueous solution of a polar organic compound, such as butyl carbitol, to animal hides permits their dehydration to give sides which are white, fibrous, and flexible. A fresh calfskin dehydrated by this method was rehydrated in a lime-unhairing bath and chrome-tanned to give a product resembling normal chrome leather. In other experiments tanning agents were added during the dehydration step to provide leathers which were completely tanned, clear-grained and flexible. Because hides and skins are perishable, dehydration if it can be accomplished economically, becomes an attractive method for preserving and stabilizing them.

Research conducted under a contract at the University of Cincinnati showed that the extraction of noncollagenous proteins from cattle hides prior to tanning gave leathers with higher tensile and grain crack strength and a finer break than control hides. The optimum conditions (primarily number of extractions and concentration of salt in the brine) were determined, and 40 hides were processed under these conditions at the East St. Louis plant of Swift and Company. The hides have now been made into leather which is undergoing evaluation.

In contract research with the Midwest Research Institute at Kansas City, Missouri, experiments are in progress to develop simplified procedures for dispersing the collagen from lime-unhaired bellies without degrading its physical and chemical properties. The new USDA trim can make available sizeable amounts of collagen from the areas of hides least desirable for leather.

C. Technology - Process and Product Development.

1. Enzymic unhairing of hides and skins. Completed studies on the enzymic unhairing of hides have demonstrated that these hides can be converted into satisfactory sole leather of commercial quality. A few lines of shoe upper leather and a crushed type handbag leather can also be made from enzyme unhaired hides without modification of tannery procedures. When enzyme unhaired hides are subjected to a brief liming treatment, additional lines of chrome-tanned shoe upper leather can be obtained. However, results between tanneries were erratic. Tanning and post-tanning operations must be adjusted to the physical conditions of the unhaired stock.

2. Abnormalities of leather. Contract research with the Tanners' Council Research Laboratory at Cincinnati, Ohio, shows that the abnormal hide condition that produces weak, mushy leather occurs most frequently in the kidney area of heavy, plump Hereford hides. This is a raw stock defect which is not caused, or significantly alleviated, by processing into leather. It is due to a vertical fibre weave in the center portion of the hide. Sorting plump, heavy Hereford hides and diverting them into sole leather, where strength is less important, is the simpliest commercial answer to the problem at present.

3. Glutaraldehyde tannage. Shearlings tanned with glutaraldehyde and basic chromium sulfate are in commercial production for use as hospital bed pads and as paint rollers. Hospital tests indicate that this tannage provides pads which are substantially more durable than alum- or chrome-tanned shearlings. This tannage has also replaced, in one firm, the tannage of shearlings with vegetable or bark tannins. Glutaraldehyde also improves the quality of work glove leather and book binding leather.

4. Regenerated collagen products. Equipment has been ordered for cutting and grinding hides and for dispersing the collagen for subsequent regeneration. Hides swollen with acids are easier to grind and the collagen easier to disperse than at a pH of 7.5-8.5. This was true for enzyme-unhaired and also for limed hides. Dispersions are being prepared from hide sections such as bellies, shoulders and splits in this attempt to find volume uses for collagen other than leather.

5. Chrome tanning. In the research under a PL-480 grant at the Lederinstitut T.N.O., Waalvijk, Holland, on the kinetics of chrome tanning, the influence of ratio of volume of tanning solution to weight of collagen on chrome retention was investigated. The effect of volume change varies with initial concentration of chrome; with chrome liquors of rather high concentration the rate of reaction and chrome fixation decreases with increasing volume ratio,

with fairly low initial concentration there is an increase of reaction velocity and chromium uptake with increasing volume ratio. The effect of basicity is more pronounced for the latter systems, especially with increasing volume ratios.

Research at Central Leather Research Institute, Madras, India, includes two other PL-480 projects. In the preliminary stages of work on radioactive tracer study of mineral tanning, methods have been established to determine ionic sulfate in chrome-tanning complexes by a scintillation technique.

Under the grant to study relationships of hide quality to tanning rate, the presence of hydroxyproline in the proteins extractable from hide pieces has been correlated with factors of deterioration such as hair slip, odor and inadequate salting. These studies show that collagen is a relatively resistant protein and that hides can be neglected for up to 20 hours before hydroxyproline becomes detectable in the extract. The noncollagenous proteins are much more susceptible to degradation as shown by a rapid rise in extractable nitrogen and tyrosine. In other studies on hides from fallen and slaughtered animals, a slighty acidic soak prior to longer liming seems to help in preventing subsequent staining of the leather due to occluded blood.

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AREA NO. 5. POTATO UTILIZATION - FOOD

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, has turned to and is becoming more and more dependent upon the development of new and improved processed products to maintain markets and avoid recurring economic disasters. Crop perishability, fluctuations in supply, and inelasticity of demand, result in wide price swings with even slight surpluses. Depressive lows are moderated by advance contracting by processors prior to harvest in producing areas having a substantial processing industry. However, in many processing areas, processing has not yet been developed and vulnerability still exists and is exaggerated by the growing competition of processed potato and other vegetable food products. If processing is to expand rapidly enough to offset progressive decline in fresh potato consumption, a continuing improvement in currently produced products and development of new products is clearly required.

Lack of adequate knowledge concerning the chemical constituents, physical properties, and enzyme systems in potatoes is limiting development of new and improved products and processing methods. Basic research on composition is needed to provide fundamental information on which an applied research program can be systematically and effectively built. Recently developed techniques make possible the isolation, characterization, and analysis of constituents responsible for flavor, color, odor, and texture of many processed food products which were not available to research in the past. Application of such techniques to potatoes and potato products should make possible the improvement of the quality of present products, both freshly processed and following storage, and provide a basis for technological and engineering studies in new product development.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of basic and applied chemical and engineering research on studies related to processing. The work of the EURDD, involving the services of chemists, biochemists, food technologists and chemical engineers at Wyndmoor, Pennsylvania, and East Grand Forks, Minnesota, is conducted in cooperation with several Agricultural Experiment Stations which supply potatoes of known cultural history and with the marketing research facilities of the Department. The chemical research program includes: isolation and characterization of the amino acid-sugar intermediate compounds responsible for the browning of chips and French-fried potatoes during processing; studies on lipids, which are believed to play an important role in storage stability of processed potato products, particularly dehydrated products; isolation and characterization of the proteins, which are important from a nutritional aspect and from their possible involvement in textural and processing characteristics; elucidation of the causes of after-cooking discoloration and isolation and characterization of the pigment formed; methods of predicting textural characteristics of potatoes for French-fried potatoes. The Eastern Division's engineering and development research program seeks to improve the quality, nutritive value and storage stability of dehydrated potato products and to develop more convenient types of

dehydrated products, such as "Instantized" pieces that rehydrate and cook quickly. The Red River Potato Processing Laboratory, East Grand Forks, Minnesota, has been established to conduct investigations relating variety and other raw material characteristics to quality of established forms of processed potatoes. This Laboratory is operated jointly by the Red River Valley Potato Growers Association, University of Minnesota, North Dakota State University, and the Agricultural Research Service.

The Federal scientific effort devoted to this area at Wyndmoor and East Grand Forks, Minnesota, totals 12.7 scientist man-years. Of this number, research on chemical composition and physical properties amounts to 8.2, research on color, texture and other quality factors amounts to 2.3, and research on technology-process and product development comprises 2.2 scientist man-years.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 15 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

1. Basic studies on potato lipids. A method has been devised for determining the absolute amount of individual fatty acids isolated from potatoes. The new technique involves addition of a known quantity of a C-17 or a C-19 standard sample with the unknown, prior to saponification and extraction. Samples of potatoes harvested as the crop matures indicate that immature potatoes contain a considerable quantity of unsaturated acids and a new acid of about 24 carbons in the chain length.

Changes in the fatty acid content have been followed from tuber formation through maturation into storage for two varieties, Kennebec and Pontiac. Unlike the behavior of oilseeds, unsaturated fatty acids in potatoes appear to reach a minimum at the normal harvest time with little change in storage. It accordingly does not presently appear necessary to propose changes in harvest time in order to bring about any lowering in the unsaturated fatty acid level.

2. Basic studies on the proteins of potatoes. Earlier observation of quantitative, but not qualitative, differences in the protein composition of different varieties of potatoes were confirmed by the application of new techniques of separating the proteins. The water-soluble proteins have been separated into three fractions by gel-filtration. The first fraction appears to contain a small peptide or peptide complex. The second consists of at least seven components, possibly all glycoproteins. The third fraction contains 15-17 additional components. An effort will be made to isolate milligram quantities for further study. Most protein components of potatoes are indicated to have molecular weights below 100,000.

3. Basic studies on the after-cooking discoloration pigment. Study of the after-cooking discolorant was continued using an extract from a batch of potatoes of known high discoloration activity. Although the discolorant can be absorbed on activated charcoal from the deproteinized aqueous solution, desorption from the column has been incomplete. It is believed that the discolorant complex is degraded in all attempts at elution employed thus far.

Confirmation of the relationship reported earlier between tuber size and stem end blackening was obtained with three additional potato samples, two samples of Pennsylvania Katahdins and one sample of Red River Valley Pontiacs. Since no correlation has been found between blackening and specific gravity, the old idea that specific gravity is related to after-cooking discoloration can now be abandoned.

4. Basic studies on reducing sugars and enzyme activity of stored potatoes.

In research at the potato processing laboratory at East Grand Forks, Minnesota, two potato varieties (Kennebec and Pontiac) were harvested at two dates, stored at 40°F., reconditioned at 65°F. and analyzed for reducing sugars, invertase activity, invertase inhibitor, phosphatase, solids and phosphorylase. During the storage, reducing sugars increased sharply and high levels of basal and total invertase developed and reached maxima after 4-6 weeks of cold storage. On continued cold storage reducing sugars remained constant, basal invertase decreased to 0, total invertase decreased significantly and an excess of invertase inhibitor developed. When the stored potatoes were reconditioned at 65°F. the reducing sugars decreased markedly, basal invertase (if present) and total invertase decreased and a large excess of invertase inhibitor developed. These changes occurred mostly during the first two weeks. Since the inhibitor action increases with temperature, the relative behavior of invertase and its associated inhibitor at different temperatures provides an explanation of the mechanism whereby reducing sugars rise and fall inversely with the temperature of potato storage.

The synthesis of invertase by aerated potato disks was demonstrated. This result, apparently caused by wounding the potato, indicates the importance of careful handling of potatoes during harvest and storage.

Phosphoglucomutase, which plays an important role in carbohydrate transformations in potatoes, has been partially purified and characterized. This enzyme preparation has an activity four hundred times higher than that previously reported for potato phosphoglucomutase.

B. Color, Texture and Other Quality Factors.

1. Texture of French-fried potatoes. A study of the effect of raw stock and processing variables on the quality of French-fried potatoes has been studied. The variables under study include specific gravity, blanch time, fresh storage time, frozen storage time and reheating time. Difficulty in obtaining sufficiently close replication of shear has now been eliminated by selecting raw materials of comparable specific gravities. Shear values

within each fraction of a potato sample of comparable specific gravity can be duplicated closely. Cooling French-fried potatoes after reheating lowers the shear of the crust because of the redistribution of moisture from the interior to the surface. This leads to flabby pieces. A preliminary investigation of microwave oven blanching and reheating suggests that microwave heating may replace the standard blanching techniques but is not well adapted to heating frozen French-fried potatoes because a soft mushy product is invariably produced.

2. Pigments formed in potato frying. An ion-exchange procedure for isolating and identifying reaction products was employed in model experiments on the reaction of amino acids and sugar under conditions employed in potato chip frying. In studies of three sugars (glucose, fructose, sucrose) with several amino acids it has been found that the reaction rates with any one sugar vary widely with the amino acid involved and that the number of products obtained also varies. For example, 12 different compounds were formed when glucose was reacted with threonine, serine or asparagine. When arginine, beta-alanine or glutamic acid are reacted with glucose only a few compounds are produced. The concentration of some products reaches a maximum early in the heating period and then decreases. These studies on model systems have shown its possibilities as a procedure for elucidating the mechanism of browning during potato chip frying.

C. Technology - Process and Product Development.

1. Quick-cooking dehydrated potato products. In the new puffing gun which employs internally-introduced superheated steam in addition to external heat, potato pieces with an original moisture content of 20-25% moisture tend to clump in the gun. This difficulty has been overcome by precoating the equilibrated pieces with 0.75% by weight of potato solids of sodium silico aluminate. Charges of up to 20 pounds weight of potato dice so treated have been exploded in the pilot plant superheated steam gun without agglomeration. This additive is rated GRAS by Food and Drug Administration.

Conditions have been determined for the commercial production of quick-cooking dehydrated potato dice and slices by this process. A publication now in press gives details and a cost estimate showing that the greatly improved products can be made for only slightly more than conventionally dried pieces.

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AREA NO. 6. VEGETABLE UTILIZATION - FOOD

Problem. Vegetable growing occupies over 3 million acres, with a yearly farm value of a billion dollars. Utilization as processed rather than fresh vegetables provides a constant source of supply with less price fluctuation. Basic compositional research is needed to provide knowledge to constituents responsible for color, flavor and texture of vegetables and the changes these constituents undergo during processing, storage, and distribution. There is also need for application of these results to developmental research on new products and new and improved processing technology. Consumer preference is shifting to "convenience" foods. An even greater emphasis on quickly prepared foods is evident in modern military feeding where high bulk density, nonrefrigerated, and rapidly rehydrating products are of primary importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program employing chemists and chemical engineers in basic and applied research on vegetable processing and products. The Federal work is conducted at Wyndmoor, Pennsylvania. The scientific effort assigned to this area totals 3.6 scientist man-years and is currently engaged in research on technology-process and product development.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 48 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Technology - Process and Product Development.

1. Quick-cooking dehydrated vegetable pieces. Engineering research on explosive-puffing of vegetables has resulted in a process for making quick-cooking carrots, beets, sweet potatoes and rutabagas. As a result of modifications in the batch-puffing gun it has become possible to greatly reduce the cycle time. These modifications include changes in design of the gun and the use of superheated steam in the operation of the gun. A cost estimate based on the improved process indicates that explosive-puffed carrot dice made in California at a raw material cost of $25 per ton can be sold for 42¢ per pound. This is 13¢ per pound less than California Vegetable Concentrates is now asking for carrot pieces made by the explosive-puffing process. Research has continued on the compression of explosion-puffed carrot pieces before final drying to increase their density to that of conventionally air-dried pieces. The dense product retains the rapid rehydration characteristics of the uncompressed product.

2. Dehydrated mushroom product. A product of excellent flavor has been made by pulping fresh mushrooms, dehydrating the slurry and grinding the dry flakes into powder. Storage tests show that the drum-dried mushrooms can be stored

in air at room temperature satisfactorily for at least nine months. The development of a simple process for making a satisfactorily mushroom powder with the characteristic flavor of the cultivated material has attracted the attention of potential processors and will help the domestic producers who are suffering from importation of canned mushrooms from Taiwan. The mushroom powder can be used in soups and sauces, or directly as a condiment in foods without prior reconstitution, and could sell as low as $3.10 to $3.30 per pound of powder.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Technology - Process and Product Development

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For related publications of State Experiment Stations on Vegetable Utilization - Food see the Summary of Current Program and Preliminary Report of Progress from the Southern Utilization Research and Development Division, Area No. 7, or Western Utilization Research and Development Division, Area No. 9.

AREA NO. 7. DECIDUOUS FRUIT AND TREE NUT UTILIZATION - FOOD

Problem. Lack of knowledge of the nature and quantities of the various chemical constituents and enzyme systems present in fresh fruits, and of the changes these undergo during processing, is a limiting factor in research designed to develop new and improved products and processing techniques. Knowledge is required on the composition and physical structure of fruits and fruit products, with emphasis on substances responsible for color and flavor, vitamins, and other constituents important in determining consumer acceptance and nutritive value of the products. Composition should be studied in relation to variety, stage of maturity, and environmental conditions of growth; and to changes occurring between harvesting and processing, during processing, and in storage and distribution. Recently developed equipment and techniques have made it possible to isolate, separate, and identify constituents that could not have been handled previously. As basic information is developed, new processing techniques will be applied in the improvement of fruit products, and in more efficient utilization of by-products from fruit processing.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, and chemical engineers engaged in both basic and applied research related to extending the use of fruits in the food processing industries. In the EU program apple products research, and investigations on the chemistry and cell structure of cherries are conducted at Wyndmoor, Pennsylvania. Development of rapidly-reconstitutable dehydrated fruit pieces is also underway at Wyndmoor. Contract research on apple texture is in progress at the Maryland Agricultural Experiment Station, College Park. Research on the metabolism of red tart cherries is being conducted under a grant at Temple University, Philadelphia. Contract research on peaches at Rutgers University, New Brunswick, has terminated.

The Federal (EU) scientific effort devoted to research in this area totals 8.6 scientist man-years. Of this number, research on chemical composition and physical properties constitutes 1.8, including 0.3 of contract research on apple texture at the Maryland Station and 0.3 under a grant at Temple University on the metabolism of red tart cherries, research on flavor amounts to 1.1, research on color, texture and other quality factors amounts to 0.3, including 0.1 of contract research on peach processing at Rutgers, and research on microbiology and toxicology amounts to 1.0. The remainder, 4.4 scientist man-years, is devoted to research on technology--process and product development.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 50 scientist man-years is devoted to this area of research.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

1. Post-harvest metabolism of cherries. The metabolism of specific C^{14}-labeled compounds injected into normal and bruised red tart cherries is being studied under grant research at Temple University. The supplies of fruit for this purpose are being carried in frozen storage. Research is under way to develop procedures for separating the various constituents of raw cherries. Alcohol extraction provides a solution which has been separated by column chromatography into fractions of sugar, organic acids and amino acids. Known procedures will be adapted for separating the alcohol insoluble fraction into components such as pectin, lignin, hemicellulose and cellulose.

2. Factors influencing apple texture. Contract research at the Maryland Agricultural Experiment Station, College Park, shows that calcium ion reacts with both pectin and hemicellulose during apple processing. The calcium content of the pectin and hemicellulose fractions was closely correlated with the firmness of the processed slices. There was also some evidence that reaction of calcium ion with cellulose may be important in fruit stored over 75 days. The finding that calcium reacts with pectin and hemicellulose provides a basis for explaining the calcium firming treatment widely used in improving texture of apple slices. Research continued on the identification of various cell wall components of raw and processed apple tissue.

B. Flavor.

Components of cherry essence. Continuing research on the separation and identification of the components of 150-fold cherry essence shows the occurrence of acetaldehyde and methanol. Other compounds have been isolated but are not yet identified. Since atmospheric fractional distillation did not excessively degrade cherry flavor, it provided a more convenient method for the concentration of the low-boiling components than did vacuum distillation. Removal of the low-boiling components provides an essence concentrate which retains some of the constituents characteristic of cherry flavor.

C. Color, Texture and Other Quality Factors.

New varieties of fruit. As a direct result of contract research (now terminated)at the New Jersey Agricultural Experiment Station, the outstanding processing quality of the Babygold series of nonmelting clingstone peaches was discovered and brought to the attention of processors. This has led to a new industry in Eastern North America.

About one million trees of the Babygold varieties have already been planted in ten states and in Canada. They will be canned as halves in western Michigan and in Virginia's Shenandoah Valley and, it is presently planned, as puree in southern Ontario, Arkansas, and the Carolinas.

A freestone peach, Red Queen, of excellent flavor and acceptable in texture when canned or frozen, will be used as a parent in the Station's peach-breeding program.

A new variety of nectarine, NJN 32, is especially promising as a source for providing a good frozen product.

Genotypes of selected new varieties of peaches and nectarines will be made available to plant breeders, growers and processors.

D. Microbiology and Toxicology.

Improved apple cider. The effective preservation of apple cider by either refrigeration or "sorbate" depends on a low initial microbial count. Either the addition of diethylpyrocarbonate to fresh apple cider or the process of irradiation of the cider with ultraviolet rays is a relatively inexpensive procedure and a promising method for destroying most of the viable microorganisms in the fresh cider.

E. Technology--Process and Product Development.

1. Explosion-puffed fruit. The improved puffing gun which makes use of superheated steam to supplement external heating has shortened the cycle and increased the capacity to a level adequate for commercial operations. Blueberries at 11-18% moisture were satisfactorily dried to 4% moisture with, however, some breakage of the berries. It was found more desirable to dry to a moisture content of 8%. With 25-pound charges and a 4-minute cycle, output at 8% moisture is about 330 pounds per hour for one puffing gun.

A 300-bushel lot of York Imperial apples has been sampled weekly for pilot plant experiments on explosive-puffing. The firmer apples taken from storage early in season show less disintegration on puffing, but rehydrate more slowly. After long storage the apples are softer and disintegrate more on puffing but rehydrate more quickly. With apples of proper firmness and with the use of superheated steam, which reduces the time required for heating in the gun to one minute, excellent pie slices and snacks have been made.

2. Processing of red tart cherries. Progress was made in the maintenance of processing quality of red tart cherries during mechanical harvesting of the 1965 crops. Mechanical harvesting of cherries has increased from 3 million pounds in 1963 to more than 25 million pounds in 1965. A development instigated by laboratory studies on bruising is retention of the freshly harvested cherries in their original orchard soak-tank while being brought to the cannery. The cherries are funneled directly from the tank into the processing line. Changes in handling and in processing line procedures to minimize bruising of the cherries are expected to provide a product of improved quality.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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For related publications of State Experiment Stations on Deciduous Fruit and Tree Nut Utilization - Food see the Summary of Current Program and Preliminary Report of Progress from the Western Utilization Research and Development Division, Area No. 7, or Southern Utilization Research and Development Division, Area No. 11.

AREA NO. 8. TOBACCO UTILIZATION - INDUSTRIAL PRODUCTS

Problem. Although neither food nor fiber, tobacco nevertheless is grown on about a million acres, and in seven states provided more farm cash receipts than any other field crop in 1964. The farm value is about $1.3 billion. This crop is unique in that it yields about $3.1 billion in Federal and State taxes. Of the problems affecting the tobacco industry, the much publicized charges concerning the effect of tobacco usage on health are the most serious. Although much controversy still surrounds these charges, the importance of the tobacco economy and the seriousness of the charges dictate that research in this area be intensified. Such a program will serve to elucidate more completely the extent of smoking-health relationships and the capabilities of research to alter the observed physiological effects of smoke on animal tissue. Information obtained in such studies may also be of value in other industrial problems, such as the determination of relationships between the chemical composition of tobacco and smoke, and the overall quality of tobacco products. It should be noted that the present program represents a significant reorientation of effort from past endeavor concerned mainly with quality problems.

USDA AND COOPERATIVE PROGRAM

The Department has an expanding program involving many facets of the chemistry and biology of tobacco and its smoke. Much of the work is basic in nature and, although the program is health-oriented, many findings of value in industrial problems not related to health may be forthcoming. The present program is divided into six general areas: basic studies on the composition of cigarette smoke; similar investigations on tobacco leaf; biochemical changes during fermentation and aging; the nature of the pyrolytic products from leaf substances or fractions; the effect of chemical additives on the composition of cigarette smoke; and biomedical studies related to the biological assaying of cigarette smoke.

The Federal scientific effort devoted to research in this area totals 20.5 scientist man-years, including 11.5 of contract research. This effort is applied as follows:

Chemical composition, physical properties and structure investigations involves 7.7 scientist man-years at Wydnmoor, Pennsylvania, on composition of cigarette smoke, oxidation products, and acids and bases in cigar smoke and 1.4 scientist man-years of contract research at Durham, North Carolina, on a study of neutral resins of tobacco leaf.

Chemical and physical investigations to improve products involves 1.3 scientist man-years of Eastern Division personnel on evaluation of cigarette modifiers at Lexington, Kentucky, under a cooperative agreement with the University of Kentucky and 7.6 scientist man-years of contract research at the University of Kentucky to study pyrolytic products, aromatic hydrocarbons

and heterocyclic bases in smoke, and improved biological assaying methods.

Research on technology process and product development involves 2.5 scientist man-years effort consisting of 1.2 under a contract with Houdry Process and Chemical Company to develop additives to modify cigarette burn temperature and 1.3 of contract research of the University of Kentucky on routine application of bioassay methods.

The contract research effort for FY 1967 will be almost doubled by the initiation of a project at Health Research Institute, Buffalo, New York, and seven more projects at the University of Kentucky.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

A total of 9 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure.

1. Composition of tobacco smoke. Cigar smoke constituents identified for the first time include farnesyl acetone, beta-phenylpropionitrile, phenylacetic acid, hydrocinnamic acid, fumaric acid, citraconic acid, meta-toluic acid and para-toluic acid. In addition the presence of para-menthene was established. The presence of benzoic, adipic and glutaric acids in cigar smoke was confirmed. Four other compounds that had been previously identified in cigarette smoke, phytol, solanone, 3,5-dimethylphenol and a methyl ethyl phenol, were also found. A new method for investigating whole smoke on a puff-by-puff basis has been developed.

Components of the neutral water-insoluble fraction of cigarette smoke condensate isolated and identified for the first time are as follows: benzyl benzoate, benzyl cinnamate, cinnamyl cinnamate, styryl cinnamate, myristicin, alpha-methylisobutyraldehyde, solanone-like compounds and a halogenated substance. Skatole and another methylindol, a dimethylindol, a trimethylindol, carbazole, a methylcarbazole and a dimethylcarbazole were also found. Myristicin, the major component of nutmeg oil, is physiologically active. This compound and cinnamyl cinnamate may be constituents of, or pyrolytic products from, a tobacco flavoring agent. The halogenated substance may originate in a pesticide. In studies of the alkylating activity of cigarette smoke (alkylation is known to be a mechanism of biological action of many mutagenic agents), efforts to reduce the variability of the procedure for determining the alkylating activity have been successful.

The isolation of myristicin represents the first compound of known biological activity to be isolated in the present tobacco program.

2. Composition of tobacco leaf. Research on the pyrolysis of high molecular weight pigments present in cured tobacco demonstrated the formation at 880°C. of at least 25 polynuclear aromatic hydrocarbons. Thirteen of these have

been identified, including benzo(a)pyrene (BaP). The latter was present at a level of about 1 mg. of BaP per gram of pigment pyrolyzed. Cigarettes to which pigment was added to double the amount of pigment naturally present in this cigarette tobacco were smoked and the BaP levels were compared with those from cigarettes without added pigment. The cigarettes with added pigment showed an average increase of 40% in BaP. It is thus clear that the pigment may contribute to the formation of BaP under actual smoking conditions. BaP is one of the two most potent carcinogens in cigarette smoke. The high molecular weight leaf pigments may prove to be a significant contributor to the polynuclear aromatic hydrocarbons of smoke.

Contract research at the Research Triangle Institute at Durham, N. C., has provided a new method for separating the components of the complicated "resin" fraction of tobacco leaf. The process involves direct removal of ketonic and hydroxy compounds from the hexane extract by successive treatment with Girard's reagent and succinic anhydride. Four different groups of substances were separated as follows: hydroxyketonic, 45%; ketonic, 30%; hydroxylic, 5% and miscellaneous neutral substances, 20%. This new method of separating the crude leaf extract may be the first significant step of an effective way to remove resins without including the accompanying low molecular weight material. The hydroxyketonic fraction contains most, if not all, of the previously unfractionatable resins.

B. Chemical and Physical Investigations to Improve Products.

Preliminary work on the evaluation of cigarette modifiers at Wyndmoor indicates that cigarette coal temperature is not significantly lowered by the addition of sodium borate, sodium tartrate or 2,2-diphenyl-1-picryhydrazyl. However, for each modifier there was noticed a significant decrease in total particulate matter of the smoke. These studies will be continued at the University of Kentucky where preparation of laboratory space is currently in progress.

Research has begun at the University of Kentucky on the use of thin-layer chromatography to establish the separation characteristics of an authentic mixture of polynuclear aromatic hydrocarbons which occur in smoke.

In research on the pyrolysis of amino acids at the University of Kentucky, at 85°C. leucine was converted almost entirely to gaseous products (97%). Lysine monohydrochloride gave a complex pyrolysate. Phenylalanine pyrolysate contained the diketopiperazine of phenylalanine. The latter compound has not been previously reported present in tobacco smoke.

Work on heterocyclic bases has begun with the separation of known heterocyclic bases with modern gas chromatographic equipment. The authentic bases under study are known constituents of tobacco or tobacco smoke.

Research on new or improved biological assay studies show that a short-term (21-30 days) bioassay system based on the appearance of lung tumors in

influenza-infected mice may be possible.

C. Technology -- Process and Product Development.

1. Modification of cigarette burn temperature. Contract research at the Houdry Process and Chemical Company showed that six additives at high (20-50%) concentrations in cigarettes gave small (less than or equal to 100°C.) depressions in burn temperature. One additive, Butox-2, a proprietary oxidative dehydrogenation catalyst of the contractor, gave a depression greater than 100°C. Seventeen other additives were ineffective or otherwise unsuitable. Relatively high concentrations of additives are required thus far to alter significantly the burn temperature of cigarettes.

2. Routine biological assay. This assay will employ the recommendations established by the Cancer Research Commission of the UICC Panel on Carcinogenicity. Two strains of mice, one with no spontaneous skin tumors and one with a low established incidence of spontaneous skin tumors, will be used to test each sample. The laboratory at the University of Kentucky, when fully operative, will test for biological activity samples that are submitted by investigators engaged in tobacco and health research at the University and in the Crops, Market Quality, and Utilization Research Divisions of the U. S. Department of Agriculture.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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Chemical and Physical Investigations to Improve Products

Henson, W. H., Jr., Johnson, W. H., and Hassler, F. J. 1965. The influence of leaf maturity and other factors on the drying rate of bright-leaf tobacco. Tobacco Science, 9, 80-84. (Ky.)

Technology -- Process and Product Development

Anon. 1965. Processing of machine-harvested stalk cut tobacco. Mass. Agr. Exp. Sta. Publ. 542. (Mass.)

Yoder, E. E., and Smith, E. M. 1965. Handling stalk-cut tobacco on portable frames. Agr. Engr., 46, 686-687. (Ky.)

Young, J. H., Bunn, J. M., and Henson, W. H., Jr. 1965. Humidity and moisture problems associated with the handling and storage of cured tobacco. Humidity and Moisture, 2, 231-238. Reinhold Pub. Co., New York, N. Y. (Ky.)

AREA NO. 9. MAPLE SAP AND SIRUP UTILIZATION - FOOD

Problem. The extensive unused stands of sugar maple trees are largely located in agriculturally depressed areas that are commonly devoted to small-scale dairy farming. Since only a small percent of the available sugar maple trees are presently tapped for sap production, and about 50% of the sirup consumed in the United States is imported, untapped sugar maples represent a good potential source of increased cash income for farmers in these areas. The maple area includes 14 states from Minnesota to Maine and south to Virginia. Under proper conditions, maple sirup can be a six-weeks seasonal crop not in competition with other farm activities and with a per acre value equal to or exceeding that of other farm products. Based largely on recent research carried out in the Department and the State Experiment Stations, the methods of collecting and processing sap into sirup are being streamlined. This has resulted in greatly increased efficiency and larger hourly returns to the sirup producer for his labor. The advent of tube collection and transportation of sap has reduced the cost of sap handling 40% and has eliminated much hand labor.

Oil-firing of evaporators and improved systems of steam removal have provided efficient and sanitary plants. The taphole germicidal pellets and sanitary methods of sap handling have tended to stabilize crop yields and standardize sirup quality. While the results of previous research have contributed to modernization of the industry, much more information is needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner. Not only can the low income farms be greatly benefited, but the existing maple industry can be put on a higher economic plane and modernized to be made competitive with other crop and livestock farming to bring about improved land use.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists and microbiologists. These scientists are engaged in both basic and applied research in investigations concerned with the problems of improving sap handling and processing, producing high-quality maple sirup, and developing new outlets for all maple products while lowering the cost of the product. Most of this work is conducted at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 3.2 scientist man-years. Of this number, research on chemical composition and physical properties comprises 1.0, research on microbiology and toxicology comprises 1.0, and research on technology--process and product development comprises 1.2, including 0.1 in contract research on sap storage with J. L. Sipple & Son, Bainbridge, New York. In the research work, cooperation is maintained with personnel of the Federal Extension Service in maple-producing states and with Cornell University.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 1.5 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

A large-scale liquid-liquid all glass apparatus was used to extract maple sirup in five-gallon batches so as to accumulate sizeable amounts of maple flavor components. These extracts were concentrated and stock-piled for subsequent gas-liquid chromatography (GLC) analysis. Concurrently different GLC techniques and packings are being investigated to effect the best separation of fractions of maple aroma and maple flavor. The data being obtained on the nature and source of maple flavor are of utmost importance in the development of improved processing methods and new maple products.

B. Microbiology and Toxicology.

Ultraviolet sterilization of sap. Ultraviolet irradiation was effective in reducing viable yeast cells in maple sap in continuous-flow systems on a laboratory scale. Stored sap exposed to ultraviolet irradiation after addition with a high count, mixed bacterial inoculum had a count of 0 at the surface after two hours of radiation at 52°F. and at the 6-inch level after 48 hours of radiation. A slower decline in bacterial count was found as the depth increased to three feet, with no indication of any increase in bacterial growth at the 3-foot level. Agitation which continuously renewed the exposed surface reduced the bacterial content throughout the sap to 100 per ml. in less than three hours and to ten per ml. in four hours.

C. Technology--Process and Product Development.

1. Maple sap storage. The control of growth of organisms in stored maple sap is important in both the farm and central evaporator plants. Preliminary experiments under commercial conditions indicate that ultraviolet irradiation of sap, prior to and during storage, plays an important role in the prevention of microbiological deterioration of stored maple sap.

2. High-flavoring process. Three large maple sirup producers used EU equipment to make trial runs on the new continuous high-flavoring process. The apparatus was also used to impart an enhanced maple flavor to blends of cane and maple sirups. General Goods Corporation, makers of "Log Cabin" sirup, subsequently initiated development of this process since it permits production of a superior blended sirup. The owners of Penick & Ford, who make "Vermont Maid" sirup, are likewise setting up continuous high-flavoring equipment. These two brands represent at least 80% of the blended sirup market.

3. Reverse osmosis. Exploratory experiments using reverse osmosis apparatus show that this technique can effectively remove at least 75% of the water

from maple sap. The concentrated maple sap is processed to maple sirup by the conventional boiling method. Sap concentrated by reverse osmosis lost none of its flavor or color producing properties, and the sirup obtained from it had full maple flavor. The reverse osmosis technique is promising as a low-cost method for concentrating maple sap.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Filipic, V. J., Underwood, J. C., and Willits, C. O. 1965. The identification of methylcylopentenolone and other compounds in maple sirup flavor extract. J. Food Sci., 30, 1008-1015.

Underwood, J. C. and Filipic, V. J. 1965. Qualitative determination of syringaldehyde and dihydroconiferyl alcohol in maple sirup. J. Assoc. Offic. Agr. Chemists, 48, 689-693.

Microbiology and Toxicology

Kissinger, John C. 1965. Progress in buddy maple sap fermentation 1956-1965. National Maple Syrup, 4, 10-11.

Kissinger, J. C. and Willits, C. O. 1966. The control of microorganisms in flowing maple sap by ultraviolet irradiation. In "Developments in Industrial Microbiology", 7, 318-325. Washington. American Institute of Biological Sciences.

Wasserman, Aaron E. 1965. Absorption and fluorescence of water-soluble pigments produced by four species of Pseudomonas. Appl. Microbiol., 13, 175-180.

Wasserman, Aaron E. and Willits, Charles O. September 7, 1965. Preparation of maple sirup from buddy sap. U. S. Patent 3,205,076.

Technology-Process and Product Development

Underwood, Joseph C., Sr. and Wasserman, Aaron E. June 22, 1965. Preparing a fluffed product from maple sugar sirup. U. S. Patent 3,190,757.

Willits, C. O., Underwood, J. C. and Stinson, E. S. 1966. A process for continuously high-flavoring of maple sirup. Food Tech.,20, 680-683.

General

Stinson, E. E. and Willits, C. O. 1965. Fractionation of the color of pure maple and other sirups by gel filtration. J. Assoc. Offic. Agr. Chemists, 48, 493-497.

Stinson, E. E. and Willits, C. O. 1965. Isolation and characterization of the high molecular weight brown colorant of maple sirup. J. Ag. & Food Chem., 13, 294-197.

Willits, C. O. 1965. New maple developments at Eastern Utilization Research and Development Division. Natl. Maple Syrup Digest, 4, 6-9.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

Color, Texture and Other Qualities

Callahan, J. C. 1965. Observations on the length of the 1963-64 maple tapping season at the Southern Indiana Forage Farm. Ind. Agr. Exp. Sta. Res. Prog. Rpt. 175, 4 p. (Ind.)

Robbins, P. W. 1965. Influence of tapping techniques on maple (Acer saccharum) sap yields. Mich. Agr. Exp. Sta. Res. Rpt. 28, 3 p. (Mich.)

AREA NO. 10. HONEY UTILIZATION - FOOD

Problem. Essential pollination of over fifty crops depends almost completely on the honeybee because of changes in agricultural practices over recent years. Most of the beekeeper's compensation, however, results not from pollination services, but from the sale of honey and wax. In spite of the essential nature of the pollination service, he is thus subject to the uncertainties of crop and weather, disease, loss of crop and bees from insecticides and herbicides, rising costs of equipment, scarcity of trained labor, in addition to a chronically depressed market for honey, with prices no higher than twenty years ago. Because of the relatively small size of operations and the scattered nature of the industry, the honey producer is out-researched, out-promoted and out-advertised by competing sweetening agents. Improved processing methods and equipment, better control of product quality, outlets for lower-grade honey, stable export markets, increased industrial use of byproducts are all needed to provide an expanding market and encourage the beekeeping industry.

USDA AND COOPERATIVE PROGRAM

The Department's honey research program at Wyndmoor, Pennsylvania, has been discontinued. The Federal scientific effort in this report period was limited to termination activities.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 1.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

1. Enzymes in honey. The presence of catalase in honey was unequivocally established for the first time. Both manometric and spectophotometric methods were employed for the catalase determinations and the results correlate well. Honey sucrase was shown to be heterogeneous.

2. Other honey constituents. Paper chromatographic studies show the presence of phosphorylated compounds, tentatively identified as glucose-6-phosphate, 2- or 3-phosphoglycerate, and α- or β-glycerophosphate. Methyl anthranilate is specific to citrus honey, with 21 samples averaging 2.87 γ/g while 12 samples of non-citrus honey showed an apparent content of only 0.07 γ/g.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Schepartz, A. I. 1965. The glucose oxidase of honey. III. Kinetics and stoichiometry of the reaction. Biochim. Biophys. Acta, 99, 161-164.

Schepartz, A. I., and Subers, M. H. 1965. A simplified method for the estimation of diastase in honey. Gleanings in Bee Culture, 93, 358-359, 378.

White, J. W., Jr. 1966. Inhibine and glucose oxidase in honey - a review. Am. Bee J., 106, 214-216.

White, J. W., Jr. 1966. Methyl anthranilate content of citrus honey. Jour. Food Sci., 31, 102-104.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

No publications reported.

AREA NO. 11. NEW CROPS UTILIZATION - INDUSTRIAL PRODUCTS

Research on new crops at the Eastern Division has been discontinued. Discussion of the problem and a description of the current Federal scientific effort on New Crops Utilization - Industrial Products is presented in the Summary of Current Program and Preliminary Report of Progress from the Northern Utilization Research and Development Division, Peoria, Illinois.

Inclusion of this area heading in the present report is for the purpose of providing continuity to publication of results of research conducted at Wyndmoor, Pennsylvania, prior to July 1, 1965.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 11 scientist man-years is devoted to this area of research.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition, Physical Properties and Structure

Lutz, D. A. and Scott, W. E. 1966. Crystal data for (-)-threo-12,13-dihydroxyoleic acid. Acta Crystallographica 20, 309.

Chemical and Physical Investigations to Improve Products

Scott, W. E. and Riser, G. R. 1966. Effect of storage temperatures on the stability of trivernolin. J. Am. Oil Chem. Soc. 43, 55-56.

Technology - Process and Product Development

Krewson, C. F. and Scott, W. E. 1966. *Euphorbia lagascae* Spreng., an abundant source of epoxyoleic acid; seed extraction and oil composition. J. Am. Oil Chem. Soc. 43, 171-174.

Krewson, C. F., Scott, W. E. and Riser, G. R. 1966. *Euphorbia* and *Vernonia* seed oil products as plasticizer-stabilizers for poly(vinyl chloride). J. Am. Oil Chem. Soc. 43, 377-379.

Riser, G. R., Riemenschneider, R. W. and Witnauer, L. P. Vernolic acid esters as plasticizers for poly(vinyl chloride). Galley proof returned to J. Am. Oil Chem. Soc. 5/18/66.

Krewson, C. F., and Scott, W. E. January 18, 1966. Process for obtaining *Vernonia anthelmintica* seed oil. U. S. Patent 3,230,239.

For related publications of State Experiment Stations on New Crops Utilization - Industrial Products see the Summary of Current Program and Preliminary Report of Progress from the Northern Utilization Research and Development Division, Area No. 13.

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress	Line Project Inc. in Area and Subheading
E6 2	Milk Products Utilization Investigations.	Wyndmoor, Pa. and Washington, D. C.		
E6 2-84*	A laboratory and pilot plant study of the effect of chemical additives on the storage of evaporated milk	Washington, D. C.	Yes	1-E-3
E6 2-85 (Rev.)	Interactions of milk proteins in solution	Wyndmoor, Pa.	Yes	1-A
E6 2-87	Methods for making fat-free and low-fat cheese	Washington, D. C.	Yes	1-E-2
E6 2-88*	Physico-chemical studies of factors influencing milk fat-plasma emulsion stability	Washington, D. C.	Yes	1-A
E6 2-93	Development of a commercially feasible process for preparing a beverage quality dry whole milk having adequate shelf life	Wyndmoor, Pa.	Yes	1-E-1
E6 2-94 (C)	Effects of nonfat dry milk on bread yeast fermentation	Madison, Wis.	Yes	1-D
E6 2-95 (C)	Increased protein stability of evaporated milk: study of calcium phosphate-casein micelles	Columbus, Ohio	Yes	1-A
E6 2-96	Improving the flavor stability of anhydrous milk fat	Washington, D. C.	Yes	1-E-4
E6 2-97*	The chemistry of bacterial spores	Washington, D. C.	Yes	1-D
E6 2-98	Studies on stale flavor in sterile milk and development of means to prevent its formation	Washington, D. C.	Yes	1-B
E6 2-99	Improved sterile whole milk concentrates: the production of reversible sol-gel transformations in high solids sterile concentrates	Washington, D. C.	Yes	1-A
E6 2-100 (C)	Removal of radioactive strontium from milk on a commercial scale	Springfield, Mo.	Yes	1-E-5
E6 2-101	Ribosomal nucleic acids	Wyndmoor, Pa.	Yes	1-A
E6 2-102 (C)	Heat stability of individual milks	St. Paul, Minn.	Yes	1-A
E6 2-103	Enzyme studies relating to milk	Wyndmoor, Pa.	Yes	1-A
E6 2-104*	Casein properties	Wyndmoor, Pa.	Yes	1-A
E6 2-105 (Gr.)	Physical changes in milk and milk concentrates associated with steam injection and bubble collapse	Raleigh, N. C.	Yes	1-A
E6 2-107 (C)	Relation of milk fat composition, particularly fat and protein, to dietary of cow	College Park, Md.	Yes	1-A
E6 2-108 (Gr.)	Flavors and their precursors in milk derived from pasture or dry feeding practices	College Park, Md.	No	
E6 2-109 (Gr.)	Study on the desirable flavors of butter: Isolation and identification of specific flavor contributing compounds and their precursors	Corvallis, Oregon	Yes	1-B
E6 2-111 (Gr.)	Lactones, methyl ketones and their precursors in milk products: effects on off-flavors and development of procedures for their control	University Park, Pa.	Yes	1-B
E6 2-113	Improvement of dried whole milk	Washington, D. C.	Yes	1-E-1
E6 2-115	New and improved processing equipment	Washington, D. C.	Yes	1-E-1
E6 2-116 (C)**	Determination of flavor and quality stability of commercial fluid milk during storage to permit Iodine-131 decay	Greenville, Ill.	No	
E6 2-117 (C)**	Determination of the commercial feasibility and practicality of a combined anion-cation fixed resin bed system for removing radionuclides from milk	Springfield, Mo.	Yes	1-E-5
E6 2-118**	Mechanisms of the development and maintenance of heat resistance and dormancy in bacterial spores	Washington, D. C.	Yes	1-D

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress	Line Project Inc. in Area and Subheading
E6 2-119**	Effects of enzymes on the chemistry, antigenicity and allergenicity of milk proteins	Washington, D. C.	No	
E6 2-120**	Identification of the principal proteins in cow's milk responsible for allergy to milk	Washington, D. C.	No	
E6 2-126**	Development of assay methods for milk allergens in experimental animals	Washington, D. C.	No	
UR-A7-(60)-5-	Milk coagulating enzymes	Karnal, Punjab,India	Yes	1-E-2
UR-A7-(60)-11	Sulfur compounds in relation to flavor and stability of milk	Karnal,Punjab,India	Yes	1-B
UR-A7-(60)-13	Buffalo milk in cheese manufacturing	Anand, India	Yes	1-E-2
UR-A7-(60)-16	Phosphoproteins of milk	Bangalore, India	Yes	1-A
UR-A7-(60)-22	Proteose-peptone fraction of milk	Karnal,Punjab,India	Yes	1-A
UR-A7-(60)-48	Dipicolinic acid synthesis in bacterial spores	Pantnagor,District Nainital, India	No	
UR-A7-(60)-105**	Starter bacteria and variants in development of cheese flavor	Karnal,Punjab,India	No	
UR-A10-(60)-37**	Formation of unnatural uncleic acids	Haifa, Israel	No	
UR-E3-(60)-7**	Structures and interactions of nucleic acid	Graz, Austria	No	
UR-E8-(60)-16	Dietary factors controlling flavor in milk	Helsinki, Finland	Yes	1-B
UR-E9-(60)-46	Nonprotein nitrogenous constituents of milk	Paris, France	Yes	1-A
UR-E9-(60)-47*	Proteolytic activity of rennin on casein	Paris, France	Yes	1-A
UR-E9-(10,60)-80	Sub-unit structure of nucleic acids	Strasbourg, France	Yes	1-A
UR-E10-(60)-3	Surface changes in fat globules of dried whole milk	Berlin, West Germany	No	
UR-E21-(60)-7*	Increasing vitamin B in cheese	Warsaw, Poland	Yes	1-E-2
UR-E21-(60)-21	Mechanisms of cheese-ripening process	Olsztyn, Poland	Yes	1-E-2
UR-E25-(60)-37	Thermal properties of milk	Madrid, Spain	No	
UR-E26-(60)-9	Methods for purification of protein complexes	Uppsala, Sweden	No	
UR-E29-(60)-41*	Studied on selected enzymes of milk	Shinfield, Reading, England	No	
UR-S3-(60)-10	Structure and properties of proteolytic enzymes	Rio de Janeiro,	Yes	1-A

* Discontinued during report year.
** Initiated during report year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in Summary of Progress	Line Project Incl. in Area and Subheading
E6 5	Meat Utilization Investigations.			
E6 5-17 (Rev.)	Chemical, physical, and biological factors involved in the development of rancidity in fats, fatty tissues, and meats	Beltsville, Md.	Yes	2-C-1
E6 5-19 (Rev.)	Studies on the recovery and identification of substances responsible for flavor and aroma in meat	Wyndmoor, Pa.	Yes	2-B
E6 5-20 (Rev.)	Chemical reactions involved in meat-curing	Wyndmoor, Pa.	Yes	2-C-2
E6 5-21(C)	A histochemical study of components of meat connective tissues and their relation to tenderness	Baton Rouge, La.	Yes	2-A
E6 5-22(Rev.)	Studies of psycrophilic microorganisms	Beltsville, Md.	Yes	2-D-1
E6 5-23	Improving the flavor of cured meats through a study of the interrelationships of temperature, curing substances, microbial metabolism and mutation rates	Beltsville, Md.	Yes	2-D-2
E6 5-24 (Rev.)	Meat protein composition and distribution in relation to tenderness and juiciness	Wyndmoor, Pa.	Yes	2-A
E6 5-26(C)	Fungi associated with meat processing and flavor development	Ames, Iowa	Yes	2-D-2
E6 5-27	Identifying substances in wood smoke that contribute to the flavor and aroma of meats	Wyndmoor, Pa.	Yes	2-B
E6 5-28	Development of new or improved meat processing methods and of new meat products	Wyndmoor, Pa.	Yes	2-E-1
E6 5-30(C)	Development of new smoked meat products	East Lansing, Michigan	Yes	2-E-2
E6 5-31(C)	New frozen meat products and their time-temperature relationships	Columbia, Mo.	Yes	2-E-2
E6 5-32(Gr.)	The relationship of amounts and ratios of heme pigments to oxidative rancidity	Tallahasse, Fla.	Yes	2-C-2
E6 5-33(Gr.)	The development of an accurate laboratory method of estimating the thermal history of meat products	Wyndmoor, Pa.	Yes	2-E-1
E6 5-34(Gr.)	A study of the nature and significance of non-carbonyl volatile compounds associated with rancidity in meats	New Brunswick, N.J.	Yes	2-C-1
E6 5-35(C)*	Development of new ready-to-eat meat products suitable for production in small, rural industries	Baton Rouge, La.	No	
E6 5-36 (C)*	Reactions of muscle proteins as they relate to the thermal effects of meat processing and large-scale institutional cookery	Ithaca, N.Y.	No	
UR-A6-(60)	New semi-dehydrated fried meat products	Taichung, Taiwan	Yes	2-E-2
UR-E8-(60)-14	Influence of fats on flavor and aroma of dry sausage	Helsinki, Finland	Yes	2-E-1
UR-E19-(60)-17	The use of protozoa to detect harmful substances in meat	Utrecht, Netherlands	No	
UR-E21 (60)-24	Antioxidant components of wood smoke used in meat-curing	Gdansk,Poland	Yes	2-B
UR-E29-(60)-70	Specific reducing systems in pork muscle	Leatherhead,Surrey, England	Yes	2-C-2

* Initiated during report year.

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
E6 3	Animal Fats and Oils and Special Products Utilization Investigations.			
E6 3-55 (Rev.)	Long-chain fat derivatives for polymer modification	Wyndmoor, Pa.	Yes	3-B-1
E6 3-58 (Rev.)	Soap-detergent combinations based on animal fats	Wyndmoor, Pa.	Yes	3-B-2
E6 3-61 (C)*	Substituted vinyl monomers and polymers	Tucson, Arizona	Yes	3-B-1
E6 3-62*	Synthetic lubricants from animal fats	Wyndmoor, Pa.	Yes	3-B-3
E6 3-64*	Structure of components and derivatives of animal fats	Wyndmoor, Pa.	Yes	3-A
E6 3-65	Fractionation and analysis of lipids	Wyndmoor, Pa.	Yes	3-A
E6 3-66	Polymerizable amides from animal fats	Wyndmoor, Pa.	Yes	3-B-1
E6 3-67 (C)*	Structural characteristics of organic peroxides	Pittsburgh, Pa.	Yes	3-A
E6 3-68*	Autoxidation of fatty materials in emulsion	Wyndmoor, Pa.	Yes	3-B-4
E6 3-69 (C)(Rev.)	Spatial interrelations within triglyceride molecules	Villanova, Pa.	Yes	3-A
E6 3-70 (C)	X-ray investigations of a mixed triglyceride	Villanova, Pa.	Yes	3-A
E6 3-71 (C)	Interfacial adsorption characteristics of salts of alkyl esters of α-sulfo fatty acids as related to their wetting and detergent action	Bethlehem, Pa.	Yes	3-A
E6 3-72	Development of industrially useful chemicals by free radical addition products	Wyndmoor, Pa.	Yes	3-B-3
E6 3-73	Biodegradable detergents from animal fats	Wyndmoor, Pa.	Yes	3-B-2
E6 3-74 (Gr)	Synthesis of pure glycerides	Storrs, Conn.	Yes	3-A
E6 3-75 (Gr)	Ozonization of animal fats	Austin, Minn.	Yes	3-B-4
E6 3-76	New mathematical approaches to physical measurement	Wyndmoor, Pa.	Yes	3-A
E6 3-77 (C)**	Additive chlorination and hydrogenolysis of animal fats	Not yet determined	No	
E6 3-78 (C)	High pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation	Chicago, Ill.	Yes	3-B-2
E6 3-80	Synthesis of chemical intermediates by the introduction of reactive ester, sulfur, and oxygen functional groups into animal fats	Wyndmoor, Pa.	Yes	3-B-4
E6 3-81 (Gr)	Biological synthesis of unsaturated fatty acids	Not yet determined	No	
E6 3-82**	Structure of animal fat constituents and derivatives by physical methods	Wyndmoor, Pa.	Yes	3-A,4-A-1
E6 3-83**	Development from animal fats of synthetic lubricants by the preparation of small ring heterocylic derivatives	Wyndmoor, Pa.	Yes	3-B-4
UR-E-21-(40,60)-28	Kinetics and thermodynamics of fat autoxidation	Gdansk, Poland	Yes	3-B-4
UR-E25-(60)-22	Cocoa butter substitutes from animal fats	Madrid, Spain	No	

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
UR-A7-(60) -72	Preparation and properties of long chain sulfated monoglycerides	Bombay, India	Yes	3-B-2
UR-E9-(60) -79	Hydroxylated fatty derivatives	Marseille, France	Yes	3-B-4
UR-E9-(60) -88	Autoxidation of fat at low temperatures	Paris, France	No	
UR-E9-(60) -89**	Polyhalogenated fatty acids and their derivatives	Paris, France	No	
UR-E21-(60) -29**	Thermally stable stationary phases for gas-liquid chromatography	Gdansk, Poland	No	

* Discontinued during report period.
** Initiated during report period.

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress	Line Project Inc. in Area and Subheading
E6 4	Hides, Skins and Leather Utilization Investigations.			
E6 4-32*	Composition and properties of animal protein residues	Wyndmoor, Pa.	No	
E6 4-34*	Processes for making commercial quality leather from enzyme-unhaired hides	Wyndmoor, Pa.	Yes	4-C-1
E6 4-36	Effect of electrolytes and lipid components on hide properties	Wyndmoor, Pa.	Yes	4-A-1
E6 4-37(C)	Noncollagenous proteins of cattlehides	Cincinnati,Ohio	Yes	4-B-3
E6 4-38 (C)(Rev.)	Preparation and properties of dispersed collagen sols	Kansas City, Missouri	Yes	4-B-3
E6 4-39	Microscopic investigation of skin and leather structure	Wyndmoor, Pa.	Yes	4-A-2
E6 4-40(C)	Abnormalities of leather characterized by a depleted mushy texture	Cincinnati,Ohio	Yes	4-C-2
E6 4-41	Physical properties of collagen and leathers	Wyndmoor, Pa.	Yes	4-A-2
E6 4-42	Addition of new reactive sites to hide proteins	Wyndmoor,Pa.	Yes	4-B-1
E6 4-43	Chemical modification of hides with aldehydes in combination with phenols, amides, hydrazides	Wyndmoor, Pa.	Yes	4-B-2
E6 4-44	Chemical modification of animal hides with cyclic urea derivatives such as urons and triazones	Wyndmoor, Pa.	Yes	4-B-2
E6 4-45	Physical properties of collagen	Evanston, Ill.	Yes	4-A-1
E6 4-46(C)**	Physical, chemical and engineering aspects of drying leather		No	
E6 4-47(C)**	New products through dispersion and reconstitution of collagen fibers		No	
E6 4-48	Regenerated collagen products for use in food products	Wyndmoor, Pa.	Yes	4-C-4
E6 4-49	Dehydration of animal hides and skins	Wyndmoor, Pa.	Yes	4-B-3
E6 4-50	New tanning processes from application of new chemical modification agents	Wyndmoor, Pa.	Yes	4-C-3
E6 4-51	Physical investigation of the fundamental factors which determine the secondary structure of collagen and other animal proteins	Wyndmoor, Pa.	Yes	4-A-1
UR-A7-(60)-17	Polyphenolic tanning compounds	Madras, India	Yes	4-B-2
UR-A7-(60)-18	Relation of hide quality to tanning rate	Madras, India	Yes	4-C-5
UR-A7-(60)-42***	Preparation and determination of the physico-chemical properties of polypeptidyl derivatives of collagen	Madras, India	No	
UR-A7-(60)-43	Hydrothermal shrinkage of collagen and leather	Madras, India	Yes	4-A-2
UR-A7-80	Radioactive tracer study of mineral tanning	Madras, India	Yes	4-C-5
UR-A7-81	The comfort properties of shoe leathers	Madras, India	Yes	4-A-2
UR-A7-92	Rapid tannage of sole leather	Madras, India	No	
UR-E8-(60)-3*	Fractionation of gelatin and collagen	Turku, Finland	Yes	4-A-1
UR-E8-(60)-17***	Basic investigations on the structure, biosynthesis and maturation of collagen	Turku, Finland	No	

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress	Inc. in Area and Subheading
R-E19-(60) 13	Kinetics of chrome tanning	Waalwijk,Holland	Yes	4-C-5
R-E29-(60) 67	Chemically reactive compounds for improving leather stability	Milton Park, Surrey, England	Yes	4-B-2

* Discontinued during report period.
** Withdrawn.
*** Initiated during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E3 6	Potato and Other Vegetable Utilizations- Eastern Region.	Wyndmoor, Pa.		
E3 6-44	Basic composition studies on the lipid fraction of potatoes	Wyndmoor, Pa.	Yes	5-A-1
E3 6-45	Basic studies on the formation and identity of the after-cooking discoloration pigment	Wyndmoor, Pa.	Yes	5-A-3
E3 6-46	Color and texture of frozen French fries	Wyndmoor, Pa.	Yes	5-B-1
E3 6-47	Effect of varietal, cultural and other source factors on the quality of processed potato products	E. Grand Forks, Minnesota	Yes	5-A-4
E3 6-48	Pilot plant investigations on methods for producing dehydrated potato pieces capable of rapid rehydration by modification of internal structure and components related to texture	Wyndmoor, Pa.	Yes	5-C-1
E3 6-49	Basic studies on proteins of potatoes	Wyndmoor, Pa.	Yes	5-A-2
E3 6-50	Pigments formed during frying of chips	Wyndmoor, Pa.	Yes	5-B-2
E3 6-41*	Development of new types of dehydrated vegetables through modification of internal structure	Wyndmoor, Pa.	Yes	6-A-1
E3 6-51**	Increased utilization of mushrooms through improved processing	Wyndmoor, Pa.	Yes	6-A-2
E3 6-52**	Pilot plant investigations of methods for producing dehydrated vegetable products in piece form capable of rapid rehydration	Wyndmoor, Pa.	No	

* Discontinued during report period.
** Initiated during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E3 3	Apples and Other Fruit Utilization Investigations-Eastern Region.	Wyndmoor, Pa.		
E3 3-32*	Rapidly-reconstitutable dried fruit products	Wyndmoor, Pa.	Yes	7-E-1
E3 3-33 (C)*	Relation of physical and chemical properties to processing characteristics of eastern peaches	New Brunswick, N. J.	Yes	7-C-1
E3 3-34	Improvement of processed cherries through studies on composition and post-harvest treatments	Wyndmoor, Pa.	Yes	7-B-1 7-E-2
E3 3-35 (C)	Relation of apple cell wall constituents to textural quality of processed products	College Park, Md.	Yes	7-A-2
E3 3-36 (Gr)	Radioactive tracer studies of cherry well wall constituents	Philadelphia, Pa.	Yes	7-A-1
E3 3-37	Development of improved cider and cider products	Wyndmoor, Pa.	Yes	7-D-1

* Discontinued during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in Summary of Progress	Line Project Incl. in Area and Subheading
E5 3	Tobacco Investigations.			
E5 3-5	Acids and bases in cigar smoke	Wyndmoor, Pa.	Yes	8-A-1
E5 3-6	Composition of cigarette smoke	Wyndmoor, Pa.	Yes	8-A-1
E5 3-7	Composition of oxidation products	Wyndmoor, Pa.	Yes	8-A-2
E5 3-8 (C) (Rev.)	Investigations of neutral resin	Durham, N. C.	Yes	8-A-2
E5 3-9 (C)	Modifications of cigarette burn temperature	Linwood, Pa.	Yes	8-C-1
E5 3-10	Evaluation of cigarette modifiers	Lexington, Ky.	Yes	8-B
E5 3-11(C)	Improved method for polynuclear hydrocarbons in smoke	Lexington, Ky.	Yes	8-B
E5 3-12(C)	Pyrolysis products of amino acids	Lexington, Ky.	Yes	8-B
E5 3-13(C)	Heterocyclic bases of cigarette smoke	Lexington, Ky.	Yes	8-B
E5 3-14(C)	Improved biological assaying	Lexington, Ky.	Yes	8-B
E5 3-14(C)	Routine biological assaying	Lexington, Ky.	Yes	8-C-2
E5 3-16 (C)*	Biological assaying of tobacco leaf, smoke and pyrolysates	Buffalo, N. Y.	No	
E5 3-17(C)*	Analysis of tobacco and smoke condensates	Lexington, Ky.	No	
E5 3-18(C)*	Production of experimental cigarettes and preparation of cigarette smoke condensates	Lexington, Ky.	No	
E5 3-19(C)*	Distribution and metabolism of benzo(a)pyrene	Lexington, Ky.	No	
E5 3-20(C)*	Electron transport system of Bacteroides melaninogenicus	Lexington, Ky.	No	
E5 3-21(C)*	Absorption of carcinogens and noncarcinogens from gastro-intestinal tract of rodents	Lexington, Ky.	No	
E5 3-22(C)*	Respiratory epithelial cell turnover			
E5 3-23(C)*	Nitrosamines in cigarette smoke	Lexington, Ky.	No	

* Initiated during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 65 to 66

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E5 1	Sugar and Sirups Investigations.	Wyndmoor, Pa.		
E5 1-74*	Chemical compounds responsible for maple flavors; precursors of maple flavor	Wyndmoor, Pa.	Yes	9-A-1,C-2
E5 1-76	Improvement of the quality of maple products through a study of the fermentation-induced biochemical reactions involved in the formation of maple color and flavor	Wyndmoor, Pa.	Yes	9-B-1
E5 1-78 (C)	Improvement of the maple industry through development of methods for prolonged storage of sap; control of microbial fermentation of sap before and after delivery to storage tanks	Bainbridge, N.Y.	Yes	9-C-1
E5 1-82**	Improvement of the maple industry through the development of more efficient and more economical methods for the concentration of maple sap to sirup	Wyndmoor, Pa.	Yes	9-C-3

* Discontinued during report period.
** Initiated during report period.